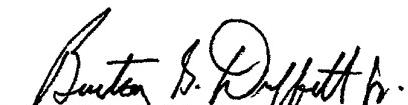


FORM-PTO-1390 (Rev. 10-98)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>				003300-685
INTERNATIONAL APPLICATION NO. PCT/SE99/00544		INTERNATIONAL FILING DATE 31 March 1999		PRIORITY DATE CLAIMED 2 April 1998 and 28 January 1999
<p><b>TITLE OF INVENTION</b> <b>AN INTEGRIN HETERO DIMER AND A SUBUNIT THEREOF</b></p> <p><b>APPLICANT(S) FOR DO/EO/US</b> <b>EVY LUNDGREN-ÅKERLUND</b></p>				
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <p>It is contemplated that this Application be prosecuted while using Claims 1 to 134 that were presented on May 29, 2000 during the international phase of prosecution as amended in the Preliminary Amendment filed herewith.</p>				
<p>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).</p> <p>*4. <input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <ol style="list-style-type: none"> <li><input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li><input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</li> </ol> <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <ol style="list-style-type: none"> <li><input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input type="checkbox"/> have been transmitted by the International Bureau.</li> <li><input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li><input type="checkbox"/> have not been made and will not be made.</li> </ol> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (signed Declaration will follow)</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11. to 16. below concern other document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information:</p> <p>Copies of Swedish Application No. 9801164-6, filed 2 April 1998 and Swedish Application No. 9900319-6, filed 28 January 1999 were submitted during the international phase of prosecution. Thus, the claim for priority has been substantiated.</p> <p>This Application qualifies for small entity status.</p>				

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) <b>09/647544</b>		INTERNATIONAL APPLICATION NO. PCT/SE99/00544		ATTORNEY'S DOCKET NUMBER 003300-685	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	PTO USE ONLY
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b> Search Report has been prepared by the EPO or JPO ..... \$860.00 (970) International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$690.00 (956) No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$710.00 (958)  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE (960)  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$100.00 (962)					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				\$ 1,000.00	
Surcharge of <b>\$130.00 (154)</b> for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$ --	
Claims	Number Filed	Number Extra	Rate		
Total Claims	134 -20 =	114	X\$18.00 (966)	\$ 2,052.00	
Independent Claims	38 -3 =	35	X\$80.00 (964)	\$ 2,800.00	
Multiple dependent claim(s) (if applicable)				\$ --	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 5,852.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$ 2,926.00	
<b>SUBTOTAL =</b>				\$ 2,926.00	
Processing fee of <b>\$130.00 (156)</b> for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). 20 <input type="checkbox"/> 30 <input type="checkbox"/> +				\$ --	
<b>TOTAL NATIONAL FEE =</b>				\$ 2,926.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$ --	
<b>TOTAL FEES ENCLOSED =</b>				\$ 2,926.00	
				Amount to be: refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/>	A check in the amount of \$ 2,926.00 to cover the above fees is enclosed.				
b. <input type="checkbox"/>	Please charge my Deposit Account No. 02-4800 in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.				
c. <input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.				
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:					
Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404			 SIGNATURE <hr/> Benton S. Duffett, Jr. NAME		
Filed: October 2, 2000			22,030 REGISTRATION NUMBER		

FORM-PTO-1390 (Rev. 10-96)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		<b>003300-685</b>	
INTERNATIONAL APPLICATION NO. <b>PCT/SE99/00544</b>		INTERNATIONAL FILING DATE <b>31 March 1999</b>	PRIORITY DATE CLAIMED <b>2 April 1998 and 28 January 1999</b>
TITLE OF INVENTION <b>AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF</b>			
APPLICANT(S) FOR DO/EO/US <b>EVY LUNDGREN-ÅKERLUND</b>			
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <p>It is contemplated that this Application be prosecuted while using Claims 1 to 134 that were presented on May 29, 2000 during the international phase of prosecution as amended in the Preliminary Amendment filed herewith.</p>			
<p>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).</p> <p>4. <input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))        a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).        b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.        c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))        a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).        b. <input type="checkbox"/> have been transmitted by the International Bureau.        c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.        d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (signed Declaration will follow)</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11. to 16. below concern other document(s) or information included:</p> <p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.  <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information:        Copies of Swedish Application No. 9801164-6, filed 2 April 1998 and Swedish Application No. 9900319-6, filed 28 January 1999 were submitted during the international phase of prosecution. Thus, the claim for priority has been substantiated.        This Application qualifies for small entity status.</p>			

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) <b>097647544</b>		INTERNATIONAL APPLICATION NO. PCT/SE99/00544	ATTORNEY'S DOCKET NUMBER 003300-685
17. <input checked="" type="checkbox"/> The following fees are submitted:		CALCULATIONS	PTO USE ONLY
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b>			
Search Report has been prepared by the EPO or JPO ..... \$860.00 (970)			
International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$690.00 (956)			
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Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE (960)			
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$100.00 (962)			
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>		\$ 1,000.00	
Surcharge of <b>\$130.00 (154)</b> for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).		20 <input type="checkbox"/> 30 <input type="checkbox"/>	\$ --
Claims	Number Filed	Number Extra	Rate
Total Claims	134 -20 =	114	X\$18.00 (966) \$ 2,052.00
Independent Claims	38 -3 =	35	X\$80.00 (964) \$ 2,800.00
Multiple dependent claim(s) (if applicable)		+ \$270.00 (968) \$ --	
<b>TOTAL OF ABOVE CALCULATIONS =</b>		\$ 5,852.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).		\$ 2,926.00	
<b>SUBTOTAL =</b>		\$ 2,926.00	
Processing fee of <b>\$130.00 (156)</b> for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)).		20 <input type="checkbox"/> 30 <input type="checkbox"/>	\$ --
<b>TOTAL NATIONAL FEE =</b>		\$ 2,926.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +		\$ --	
<b>TOTAL FEES ENCLOSED =</b>		\$ 2,926.00	
		Amount to be: refunded \$	
		charged \$	
a. <input checked="" type="checkbox"/> A check in the amount of \$ 2,926.00 to cover the above fees is enclosed.			
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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.			
SEND ALL CORRESPONDENCE TO:			
Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404		 SIGNATURE <u>Benton S. Duffett, Jr.</u> NAME	
Filed: October 2, 2000		<u>22,030</u> REGISTRATION NUMBER	

09/647544

528 Rec'd PCT/PTO 02 OCT 2000

Patent  
Attorney's Docket No. 003300-685

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of ) BOX PCT  
EVY LUNDGREN-ÅKERLUND ) Attention: DO/EO/US  
)  
Application No.: Unassigned ) Group Art Unit: Unassigned  
)  
Filed: October 2, 2000 ) Examiner: Unassigned  
)  
For: AN INTEGRIN HETERO DIMER )  
AND A SUBUNIT THEREOF )  
)  
)

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

This application corresponds to International Application No. PCT/SE99/00544,  
filed March 31, 1999.

It is contemplated that this Application be prosecuted while using Claims 1 to 134  
that were submitted on May 29, 2000 during the international phase of prosecution as  
further amended herein.

**In the Abstract:**

Please add the Abstract of the Disclosure that is provided on a separate sheet.

In the Claims:

Claim 8, lines 1 and 2, delete "in any one of claims 6 and 7" and insert --claim 6--.

Claim 20, line 1, delete "or 19".

Claim 21, line 1, delete "or 19".

Claim 26, line 2, delete "any one of claims 22-25" and insert --claim 22--.

Claim 27, lines 2 and 3, delete "any one of claims 22-25" and insert --claim 22--.

Claim 28, line 3, delete "any one of claims 22-25" and insert --claim 22--.

Claim 38, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 41, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 42, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 43, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 44, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 45, line 1, delete "any one of claims 31-37 and insert --claim 31--.

Claim 52, lines 1 and 2, delete "any one of claims 46-51" and insert --claim 46--.

Claim 53, lines 1 and 2, delete "any one of claims 46-51" and insert --claim 46--.

Claim 60, lines 1 and 2, delete "any one of claims 54-59" and insert --claim 54--.

Claim 72, lines 1 and 2, delete "any one of claims 64-71" and insert --claim 64--.

Claim 93, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 96, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 97, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 98, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 105, lines 1 and 2, delete "any one of claims 99-104" and insert --claim 99--.

Application No. Unassigned  
Attorney's Docket No. 003300-685  
Page 4

Claim 106, lines 1 and 2, delete "any one of claims 99-104" and insert --claim  
99--.

Claim 113, lines 1 and 2, delete "any one of claims 107-112" and insert --claim  
107--.

Claim 125, lines 1 and 2, delete "any one of claims 117-124" and insert --claim  
117--.

**REMARKS**

The present Amendment adds an Abstract of the Disclosure on a separate sheet and eliminates the use of multiple dependency.

The examination and allowance of the application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: *Benton S. Duffett Jr.*  
Benton S. Duffett, Jr.  
Registration No. 22,030

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Date: October 2, 2000

Abstract of the Disclosure

A recombinant or isolated integrin heterodimer comprising a novel subunit  $\alpha 10$  in association with a subunit  $\beta$  is described. The  $\alpha 10$  integrin may be purified from bovine chondrocytes on a collagen-type-II affinity column. The integrin or the subunit  $\alpha 10$  can be used as marker or target of all types of cells, e.g. of chondrocytes, osteoblasts and fibroblasts. The integrin or subunit  $\alpha 10$  thereof can be used as marker or target in different physiological or therapeutic methods. They can also be used as active ingredients in pharmaceutical compositions and vaccines.

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J9/647544

Patent

Attorney's Docket No. 003300-685

Applicant or Patentee: EVY LUNDGREN-ÅKERLUND

Application or Patent No.: \_\_\_\_\_

Filed or Issued: October 26, 2000For: AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 C.F.R. §§ 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

[ ]

the owner of the small business concern identified below:  
an official of the small business concern empowered to act on behalf of the concern  
identified below:

NAME OF CONCERN CARTELA ABADDRESS OF CONCERN c/o Evy Lundgren-ÅkerlundTrollsjövägen 165, 237 33 Bjärred, Sweden

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average, over the previous fiscal year of the concern, of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled An integrin heterodimer and a subunit thereof

by inventor(s) Evy Lundgren-Åkerlund  
described in

[ ] the specification filed herewith  
 [X] Application No. PCT/SE99/00544, filed 31 March 1999  
 [ ] Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern, or organization having rights to the invention is listed below,\* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c), or by any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

Application Serial No. \_\_\_\_\_  
Attorney's Docket No. 003300-685

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

individual     small business concern     nonprofit organization

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

individual     small business concern     nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee and any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING ENY LUNDAREN - AKERLUND

TITLE OF PERSON OTHER THAN OWNER MANAGING DIRECTOR

ADDRESS OF PERSON SIGNING Trollsjövägen 165  
237 33 BJÄRRED, SWEDEN

SIGNATURE Eny Lundaren Akerlund DATE 2000-10-09

AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

#### FIELD OF THE INVENTION

The present invention relates to a recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , the subunit  $\alpha 10$  thereof, homologues and fragments of said integrin and of said subunit  $\alpha 10$  having similar biological activity, processes of producing the same, polynucleotides and oligonucleotides encoding the same, vectors and cells comprising the same, binding entities binding specifically to the same, and the use of the same.

#### BACKGROUND OF THE INVENTION

The integrins are a large family of transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions (1-5). All known members of this superfamily are non-covalently associated heterodimers composed of an  $\alpha$ - and a  $\beta$ -subunit. At present, 8  $\beta$ -subunits ( $\beta 1-\beta 8$ ) (6) and 16  $\alpha$ -subunits ( $\alpha 1-\alpha 9$ ,  $\alpha V$ ,  $\alpha M$ ,  $\alpha L$ ,  $\alpha X$ ,  $\alpha IIb$ ,  $\alpha E$  and  $\alpha D$ ) have been characterized (6-21), and these subunits associate to generate more than 20 different integrins. The  $\beta 1$ -subunit has been shown to associate with ten different  $\alpha$ -subunits,  $\alpha 1-\alpha 9$  and  $\alpha V$ , and to mediate interactions with extracellular matrix proteins such as collagens, laminins and fibronectin. The major collagen binding integrins are  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  (22-25). The integrins  $\alpha 3\beta 1$  and  $\alpha 9\beta 1$  have also been reported to interact with collagen (26,27) although this interaction is not well understood (28). The extracellular N-terminal regions of the  $\alpha$  and  $\beta$  integrin subunits are important in the binding of ligands (29,30). The N-terminal region of the  $\alpha$ -subunits is composed of a seven-fold repeated sequence (12,31) containing FG and GAP consensus sequences. The repeats are predicted to fold into a  $\beta$ -propeller domain

(32) with the last three or four repeats containing putative divalent cation binding sites. The  $\alpha$ -integrin subunits  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha D$ ,  $\alpha E$ ,  $\alpha L$ ,  $\alpha M$  and  $\alpha X$  contain a ~200 amino acid inserted domain, the I-domain (A-domain), which 5 shows similarity to sequences in von Willebrand factor, cartilage matrix protein and complement factors C2 and B (33,34). The I-domain is localized between the second and third FG-GAP repeats, it contains a metal ion-dependent adhesion site (MIDAS) and it is involved in binding of 10 ligands (35-38).

Chondrocytes, the only type of cells in cartilage, express a number of different integrins including  $\alpha 1\beta 1$ ,  $\alpha 2\beta 1$ ,  $\alpha 3\beta 1$ ,  $\alpha 5\beta 1$ ,  $\alpha 6\beta 1$ ,  $\alpha v\beta 3$ , and  $\alpha v\beta 5$  (39-41). It has been shown that  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  mediate chondrocyte interactions with collagen type II (25) which is one of the major components in cartilage. It has also been shown 15 that  $\alpha 2\beta 1$  is a receptor for the cartilage matrix protein chondroadherin (42).

## 20 SUMMARY OF THE INVENTION

The present invention relates to a novel collagen type II binding integrin, comprising a subunit  $\alpha 10$  in association with a subunit  $\beta$ , especially subunit  $\beta 1$ , but also other  $\beta$ -subunits may be contemplated. In preferred 25 embodiments, this integrin has been isolated from human or bovine articular chondrocytes, and human chondrosarcoma cells.

The invention also encompasses integrin homologues of said integrin, isolated from other species, such as 30 bovine integrin heterodimer comprising a subunit  $\alpha 10$  in association with a subunit  $\beta$ , preferably  $\beta 1$ , as well as homologues isolated from other types of human cells or from cells originating from other species.

The present invention relates in particular to a 35 recombinant or isolated integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and or fragments thereof having the

same biological activity.

- The invention further relates to a process of producing a recombinant integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having similar biological activity, which process comprises the steps of
- 5 a) isolating a polynucleotide comprising a nucleotide sequence coding for a integrin subunit  $\alpha 10$ , or homologues or fragments thereof having similar biological
  - 10 activity,
  - b) constructing an expression vector comprising the isolated polynucleotide,
  - c) transforming a host cell with said expression vector,
  - 15 d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit  $\alpha 10$ , or homologues or fragments thereof having similar biological activity, in said transformed host cell, and, optionally,
  - 20 e) isolating the integrin subunit  $\alpha 10$ , or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.

The integrin subunit  $\alpha 10$ , or homologues or fragments thereof having the same biological activity, can also be provided by isolation from a cell in which they are naturally present.

The invention also relates to an isolated polynucleotide comprising a nucleotide coding for a integrin subunit  $\alpha 10$ , or homologues or fragments thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or parts thereof.

The invention further relates to an isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA encoding an integrin subunit  $\alpha 10$ , having the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof, wherein said polyoligo-

nucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding the integrin subunit  $\alpha 1$ .

The invention relates in a further aspect to vectors comprising the above polynucleotides, and to cells containing said vectors and cells that have polynucleotides or oligonucleotides as shown in SEQ ID No. 1 or 2 integrated in their genome.

The invention also relates to binding entities having the capability of binding specifically to the integrin subunit  $\alpha 10$  or to homologues or fragments thereof, such as proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies or monoclonal antibodies.

In a further aspect, the invention relates to a recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having similar biological activity.

In a preferred embodiment thereof, the subunit  $\beta$  is  $\beta 1$ .

The invention also relates to a process of producing a recombinant integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit  $\alpha 10$  of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof having similar biological activity,

b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit  $\alpha 10$  in combination with an expression vector comprising said isolated nucleotide coding for said subunit  $\beta$ ,

- c) transforming a host cell with said expression vectors,
- d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-unit  $\beta$ , or homologues or fragments thereof similar biological activity, in said transformed host cell, and, 5 optionally,
- e) isolating the integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.
- 10 The integrin heterodimer, or homologues or fragments thereof having similar biological activity, can also be provided by isolation from a cell in which they are naturally present.
- 15 The invention further relates to a cell containing a first vector, said first vector comprising a polynucleotide coding for a subunit  $\alpha 10$  of an integrin heterodimer, or for homologues or parts thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and, optionally, a second vector, said second vector comprising a polynucleotide coding for a 20 subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof.
- 25 In still another aspect, the invention relates to binding entities having the capability of binding specifically to the integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, preferably wherein the subunit  $\beta$  is  $\beta 1$ . Preferred binding entities are proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies and monoclonal antibodies.
- 30 In a further aspect, the invention relates to a fragment of the integrin subunit  $\alpha 10$ , which fragment is 35 a peptide chosen from the group comprising peptides of

the cytoplasmic domain, the I-domain and the spliced domain.

In one embodiment, said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

5 In another embodiment, said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

10 In a further embodiment, said fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 in SEQ ID No. 1.

Another embodiment of the invention relates to a polynucleotide or oligonucleotide coding for a fragment of the human integrin subunit  $\alpha 10$ . In one embodiment this polynucleotide or oligonucleotide codes for a fragment 15 which is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain. In further embodiments the polynucleotide or oligonucleotide codes for the fragments defined above.

The invention also relates to binding entities having the capability of binding specifically to a fragment 20 of the integrin subunit  $\alpha 10$  as defined above.

The invention also relates to a process of using an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin 25 heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or a homologue or fragment of said integrin or subunit having similar biological activity, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including 30 human origin.

In an embodiment of this process the fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

35 In further embodiments of said process the fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid no. 952 to

about amino acid No. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID no. 1.

The subunit  $\beta$  is preferably  $\beta 1$ . The cells are preferably chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

Said process may be used during pathological conditions involving said subunit  $\alpha 10$ , such as pathological conditions comprising damage of cartilage, or comprising trauma, rheumatoid arthritis and osteoarthritis.

Said process may be used for detecting the formation of cartilage during embryonal development, or for detecting physiological or therapeutic reparation of cartilage.

Said process may also be used for selection and analysis, or for sorting, isolating or purification of chondrocytes.

A further embodiment of said process is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

A still further embodiment of said process is a process for in vitro studies of differentiation of chondrocytes.

The invention also comprises a process of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

The fragment in said process may be a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain. In preferred embodiments said fragment is a peptide comprising the

amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid No. 952 to about amino acid No. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about 5 amino acid No. 140 to about amino acid no. 337 of SEQ ID No. 1.

The process may also be used for detecting the presence of an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or 10 of an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or of homologues or fragments thereof having similar biological activity.

In a further embodiment said process is a process for determining the differentiation-state of cells during 15 embryonic development, angiogenesis, or development of cancer.

In a still further embodiment this process is a process for detecting the presence of an integrin sub-unit  $\alpha 10$ , or of a homologue or fragment of said integrin 20 subunit having similar biological activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions where- 25 in said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ . Said cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts. Said integrin fragment may 30 be a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain, such as a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid no. 952 to 35 about amino acid no. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of SEQ ID No. 1.

In a still further embodiment the process is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage. The pathological conditions may be any pathological conditions involving the integrin subunit  $\alpha 10$ , such as rheumatoid arthritis, osteoarthritis or cancer. The cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, 10 osteoblasts and fibroblasts.

The invention also relates to a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit 15  $\alpha 1$ . Embodiments of this aspect comprise a process, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain, 20 such as a polynucleotide or oligonucleotide coding for a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or comprising the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1, or the amino acid sequence 25 from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1. Said pathological conditions may be any pathological conditions involving the integrin subunit  $\alpha 10$ , such as rheumatoid arthritis, osteoarthritis or cancer, or atherosclerosis or inflammation. Said cells 30 may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

In a further aspect the invention relates to a pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity, as a target molecule. An embodiment of said pharmaceutical composition is intended for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels. A further embodiment comprises a pharmaceutical composition for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

The invention is also related to a vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$ , or DNA or RNA coding for said integrin subunit  $\alpha 10$ .

A further aspect of the invention is related to the use of the integrin subunit  $\alpha 10$  as defined above as a marker or target in transplantation of cartilage or chondrocytes.

A still further aspect of the invention is related to a method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

The invention is also related to the use of an integrin subunit  $\alpha 10$  or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$  as a target for anti-

adhesive drugs or molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

The invention also relates to a method of stimulating, inhibiting or blocking the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity, as a target molecule.

In another embodiment the invention is related to a method of preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using a integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity, as a target molecule.

The invention also relates to a method of stimulating extracellular matrix synthesis and repair by activation or blockage of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or of the subunit  $\alpha 10$  thereof, or of a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity.

In a further aspect the invention relates to a method of in vitro detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, with a sample, thereby causing said integrin, subunit  $\alpha 10$ , or homologue or fragment thereof having similar biological activity, to modulate

the binding to its natural ligand or other integrin binding proteins present in said sample.

The invention also relates to a method of in vitro studying consequences of the interaction of a human heterodimer integrin comprising a subunit  $\alpha 10$  and a sub-unit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, with an integrin binding entity and thereby initiate a cellular reaction. Said consequences may be measured as alterations in cellular functions.

A still further aspect of the inventions relates to a method of using DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof as a molecular target. In an embodiment of this aspect, a polynucleotide or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof, whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

The invention also relates to a method of using a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof, as a marker or target molecule during angiogenesis.

#### BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 Affinity purification of the  $\alpha 10$  integrin sub-unit on collagen type II-Sepharose.

Fig. 2. Amino acid sequences of peptides from the bovine  $\alpha 10$  integrin subunit.

Fig. 3a. Affinitypurification and immunoprecipitation of the integrin subunit  $\alpha 10$  from bovine chondrocytes.

Fig. 3b. Affinitypurification and immunoprecipitation of the integrin subunit  $\alpha 10$  from human chondrocytes.

Fig. 3c. Affinitypurification and immunoprecipitation of the integrin subunit  $\alpha 10$  from human chondrosarcoma cells.

5 Fig. 4. A 900 bp PCR-fragment corresponding to the bovine integrin subunit  $\alpha 10$

Fig. 5. Schematic map of the three overlapping  $\alpha 10$  clones.

Fig. 6. Nucleotide sequence and deduced amino acid sequence of the human  $\alpha 10$  integrin subunit.

10 Fig. 7. Northern blot of integrin  $\alpha 10$  mRNA.

Fig. 8 Immunoprecipitation of the  $\alpha 10$  integrin subunit from human chondrocytes using antibodies against the cytoplasmic domain of  $\alpha 10$  (a). Western blot of the  $\alpha 10$  associated  $\beta$ -chain (b).

15 Fig. 9. Immunostaining of  $\alpha 10$  integrin in human articular cartilage.

Fig. 10 Immunostaining of  $\alpha 10$  integrin in 3 day mouse limb cartilage.

20 Fig 11. Immunostaining of  $\alpha 10$  integrin in 13.5 day mouse embryo.

Fig 12. Hybridisation of  $\alpha 10$  mRNA in various human tissues.

25 Fig. 13 Immunostaining of fascia around tendon (a), skeletal muscle (b) and heart valves (c) in 3 day mouse limb.

Fig. 14. PCR fragments corresponding to  $\alpha 10$  integrin subunit from human chondrocytes, human endothelial cells, human fibroblasts and rat tendon.

30 Fig 15. Partial genomic nucleotide sequence of the human integrin subunit  $\alpha 10$ .

Fig 16. Upregulation of  $\alpha 10$  integrin subunit in chondrocytes cultured in alginate.

Fig 17. Immunoprecipitation of the  $\alpha 10$  integrin subunit from human smooth muscle cells

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#### DETAILED DESCRIPTION OF THE INVENTION

The present invention demonstrate that human and

bovine chondrocytes express a novel, collagen type II-binding integrin in the  $\beta 1$ -family. An earlier study presented some evidence for that human chondrosarcoma cells also express this integrin (25). Immunoprecipitation experiments using antibodies against the integrin subunit  $\beta 1$  revealed that this novel  $\alpha$ -integrin subunit had an apparent molecular weight ( $M_r$ ) of approximately 160 kDa under reducing conditions, and was slightly larger than the  $\alpha 2$  integrin subunit. To isolate this  $\alpha$ -subunit collagen type II-binding proteins were affinity purified from bovine chondrocytes. The chondrocyte lysate was first applied to a fibronectin-Sepharose precolumn and the flow through was then applied to a collagen type II-Sepharose column. A protein with  $M_r$  of approximately 160 kD was specifically eluted with EDTA from the collagen column but not from the fibronectin column. The  $M_r$  of this protein corresponded with the  $M_r$  of the unidentified  $\beta 1$ -related integrin subunit. The 160 kD protein band was excised from the SDS-PAGE gel, digested with trypsin and the amino acid sequences of the isolated peptides were analysed.

Primers corresponding to isolated peptides amplified a 900 bp PCR-fragment from bovine cDNA which was cloned, sequenced and used for screening of a human articular chondrocyte  $\lambda$ ZapII cDNA library to obtain the human integrin  $\alpha$ -subunit homologue. Two overlapping clones, hc1 and hc2 were isolated, subcloned and sequenced. These clones contained 2/3 of the nucleotide sequence including the 3' end of the cDNA. A third clone which contained the 5'end of the  $\alpha 10$  cDNA, was obtained using the RACE technique. Sequence analysis of the 160 kD protein sequence showed that it was a member of the integrin  $\alpha$ -subunit family and the protein was named  $\alpha 10$ .

The deduced amino acid sequence of  $\alpha 10$  was found to share the general structure of the integrin  $\alpha$ -subunits described in previously published reports (6-21). The large extracellular N-terminal part of  $\alpha 10$  contains a

seven-fold repeated sequence which was recently predicted to fold into a  $\beta$ -propeller domain (32). The integrin subunit  $\alpha 10$  contains three putative divalent cation-binding sites (DxD/NxD/NxxxD) (53), a single spanning transmembrane domain and a short cytoplasmic domain. In contrast to most  $\alpha$ -integrin subunits the cytoplasmic domain of  $\alpha 10$  does not contain the conserved sequence KxGFF (R/K) R. The predicted amino acid sequence in  $\alpha 10$  is KLGFFAH. Several reports indicate that the integrin cytoplasmic domains are crucial in signal transduction (54) and that membrane-proximal regions of both  $\alpha$ - and  $\beta$ -integrin cytoplasmic domains are involved in modulating conformation and affinity state of integrins (55-57). It is suggested that the GFFKR motif in  $\alpha$ -chains are important for association of integrin subunits and for transport of the integrin to the plasma membrane (58). The KxGFFKR domain has been shown to interact with the intracellular protein calreticulin (59) and interestingly, calreticulin-null embryonic stem cells are deficient in integrin-mediated cell adhesion (60). It is therefore possible that the sequence KLGFFAH in  $\alpha 10$  have a key function in regulating the affinity between  $\alpha 10\beta 1$  and matrix proteins.

Integrin  $\alpha$  subunits are known to share an overall identity of 20-40% (61). Sequence analysis showed that the  $\alpha 10$  subunit is most closely related to the I-domain containing  $\alpha$ -subunits with the highest identity to  $\alpha 1$  (37%) and  $\alpha 2$  (35%). The integrins  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  are known receptors for both collagens and laminins (24;62;63) and we have also recently demonstrated that  $\alpha 2\beta 1$  interacts with the cartilage matrix protein chondroadherin (42). Since  $\alpha 10\beta 1$  was isolated on a collagen type II-Sepharose, we know that collagen type II is a ligand for  $\alpha 10\beta 1$ . We have also shown by affinity purification experiments that  $\alpha 10\beta 1$  interacts with collagen type I but it remains to be seen whether laminin or chondroadherin are also ligands for this integrin.

The  $\alpha 10$  associated  $\beta$ -chain migrated as the  $\beta 1$  integrin subunit both under reducing and non-reducing conditions. To verify that the  $\alpha 10$  associated  $\beta$ -chain indeed is  $\beta 1$ , chondrocyte lysates were immunoprecipitated with 5 antibodies against  $\alpha 10$  or  $\beta 1$  followed by Western blot using antibodies against the  $\beta 1$ -subunit. These results clearly demonstrated that  $\alpha 10$  is a member of the  $\beta 1$ -integrin family. However, the possibility that  $\alpha 10$  combine also with other  $\beta$ -chains can not be excluded..

10 A polyclonal peptide antibody raised against the cytoplasmic domain of  $\alpha 10$  precipitated two protein bands with  $M_r$  of approximately 160 kD ( $\alpha 10$ ) and 125 kD ( $\beta 1$ ) under reducing conditions. Immunohistochemistry using the  $\alpha 10$ -antibody showed staining of the chondrocytes in tissue sections of human articular cartilage. The antibody staining was clearly specific since preincubation of the antibody with the  $\alpha 10$ -peptide completely abolished the staining. Immunohistochemical staining of mouse limb sections from embryonic tissue demonstrated that  $\alpha 10$  is 15 upregulated during condensation of the mesenchyme. This indicate that the integrin subunit  $\alpha 10$  is important during the formation of cartilage. In 3 day old mice  $\alpha 10$  was found to be the dominating collagen binding integrin subunit which point to that  $\alpha 10$  has a key function in 20 maintaining normal cartilage functions.

25 Expression studies on the protein and mRNA level show that the distribution of  $\alpha 10$  is rather restrictive. Immunohistochemistry analyses have shown that  $\alpha 10$  integrin subunit is mainly expressed in cartilage but it is 30 also found in perichondrium, periosteum, ossification groove of Ranvier, in fascia surrounding tendon and skeletal muscle and in the tendon-like structures in the heart valves. This distribution point to that  $\alpha 10$  integrin subunit is present also on fibroblasts and 35 osteoblasts. PCR amplification of cDNA from different cell types revealed the presence of an alternatively spliced  $\alpha 10$  integrin subunit. This spliced  $\alpha 10$  was domi-

nating in fibroblasts which suggests that  $\alpha 10$  in fibroblasts may have a different function compared to  $\alpha 10$  present on chondrocytes.

5 Expression of the integrin subunit  $\alpha 10$  was found to decrease when chondrocytes were cultured in monolayer. In contrast, the expression of  $\alpha 10$  was found to increase when the cells were cultured in alginate beads. Since the latter culturing model is known to preserve the phenotype of chondrocytes the results suggest that  $\alpha 10$  can function  
10 as marker for a differentiated chondrocyte.

Adhesion between tendon/ligaments and the surrounding tissue is a well-known problem after infection, injury and after surgical intervention. Adhesion between tendon and tendon sheets impairs the gliding function and  
15 cause considerable problems especially during healing of tendons in e.g. the hand and fingers leading to functional incapacity. The localisation of the  $\alpha 10$  integrin subunit in the fascia of tendon and skeletal muscle makes  $\alpha 10$  a possible target for drugs and molecules with anti-adhesive properties that could prevent impairment of the  
20 function of tendon/ligament. The integrin subunit  $\alpha 10$  can also be a target for drugs or molecules with anti-adhesive properties in other tissues where adhesion is a problem.

25

#### EXAMPLES

##### Example 1

Affinity purification of the  $\alpha 10$  integrin subunit on  
30 collagen type II-Sepharose.

##### Materials and Methods

Bovine chondrocytes, human chondrocytes or human chondrosarcoma cells were isolated as described earlier [Holmwall et al, Exp Cell Res, 221, 496-503 (1995),  
35 Camper et al, JBC, 273, 20383-20389 (1998)]. A Triton X-100 lysate of bovine chondrocytes was applied to a fibronectin-Sepharose precolumn followed by a collagen

type II-Sepharose column and the integrin subunit  $\alpha 10$  was eluted from the collagen type II-column by EDTA (Camper et al, JBC, 273, 20383-20389 (1998)). The eluted proteins were precipitated by methanol/chloroform, separated by SDS-PAGE under reducing conditions and stained with Coomassie blue. (Camper et al, JBC, 273, 20383-20389 (1998)). Peptides from the  $\alpha 10$  protein band were isolated by in-gel digestion with a trypsin and phase liquid chromatography and sequenced by Edman degradation (Camper et al, JBC, 273, 20383-20389 (1998)).

#### Results

Fig 1 shows EDTA-eluted proteins from the fibronec-tin-Sepharose (A), flow-through from the collagen type II-Sepharose column (B) and EDTA-eluted proteins from the collagen type II-Sepharose (C). The  $\alpha 10$  integrin subunit (160 kDa) which was specifically eluted from the collagen type II column is indicated with an arrow. Figure 2 shows the amino acid sequences of six peptides that were isolated from the bovine integrin subunit  $\alpha 10$ . Figures 3 a, b, and c show that the  $\alpha 10$  integrin subunit is present on bovine chondrocytes (3a), human chondrocytes (3b) and human chondrosarcoma cells (3c). The affinity for collagen type II, the coprecipitation with  $\beta 1$ -integrin subunit and the molecular weight of 160 kDa under reducing conditions identify the  $\alpha 10$  integrin subunit on the different cells. These results show that  $\alpha 10$  can be isolated from chondrocytes and from chondrosarcoma cells.

#### Example 2

30 Amplification of PCR fragment corresponding to bovine  $\alpha 10$  integrin subunit.

#### Materials and methods

The degenerate primers GAY AAY ACI GCI CAR AC (DNTAQAT, forward) and TIA TIS WRT GRT GIG GYT (EPHHSI, 35 reverse) were used in PCR (Camper et al, JBC, 273, 20383-20389 (1998)) to amplify the nucleotide sequence corresponding to the bovine peptide 1 (Figure 2). A 900 bp

PCR-fragment was then amplified from bovine cDNA using an internal specific primer TCA GCC TAC ATT CAG TAT (SAYIQY, forward) corresponding to the cloned nucleotide sequence of peptide 1 together with the degenerate primer ICK RTC  
5 CCA RTG ICC IGG (PGHWDR, reverse) corresponding to the bovine peptide 2 (Figure 2). Mixed bases were used in positions that were twofold degenerate and inosines were used in positions that are three- or fourfold degenerate. mRNA isolation and cDNA synthesis was done as earlier  
10 described (Camper et al, JBC, 273, 20383-20389 (1998)). The purified fragment was cloned, purified and sequenced as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)).

#### Results

15 The nucleotide sequence of peptide 1 (Figure 2) was obtained by PCR-amplification, cloning and sequencing of bovine cDNA. From this nucleotide sequence an exact primer was designed and applied in PCR-amplification with degenerate primers corresponding to peptides  
20 2-6 (Figure 2). Primers corresponding to peptides 1 and 2 amplified a 900 bp PCR-fragment from bovine cDNA (Figure 4).

#### Example 3

25 Cloning and sequence analysis of the human  $\alpha 10$  integrin subunit

#### Material and methods

The cloned 900bp PCR-fragment, corresponding to bovine  $\alpha 10$ -integrin, was digoxigenin-labelled according  
30 to the DIG DNA labelling kit (Boehringer Mannheim) and used as a probe for screening of a human articular chondrocyte  $\lambda$ ZapII cDNA library (provided by Michael Bayliss, The Royal Veterinary Basic Sciences, London, UK) (52). Positive clones containing the pBluescript SK+ plasmid  
35 with the cDNA insert were rescued from the ZAP vector by *in vivo* excision as described in the ZAP-cDNA® synthesis kit (Stratagene). Selected plasmids were purified and

20

sequenced as described earlier (Camper et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and internal specific primers. To obtain cDNA that encoded the 5' end of  $\alpha 10$  we designed the primer AAC TCG TCT TCC AGT GCC ATT CGT GGG 5 (reverse; residue 1254-1280 in  $\alpha 10$  cDNA) and used it for rapid amplification of the cDNA 5' end (RACE) as described in the Marathon™ cDNA Amplification kit (Clontech INC., Palo Alto, CA).

#### Results

10 Two overlapping clones, hc1 and hc2 (Figure 5), were isolated, subcloned and sequenced. These clones contained 2/3 of the nucleotide sequence including the 3' end of the cDNA. A third clone (racel; Figure 5), which contained the 5' end of the  $\alpha 10$  cDNA, was obtained using the RACE technique. From these three overlapping clones of 15  $\alpha 10$  cDNA, 3884 nucleotides were sequenced. The nucleotide sequence and deduced amino acid sequence is shown in Figure 6. The sequence contains a 3504-nucleotide open reading frame that is predicted to encode a 1167 amino 20 acid mature protein. The signal peptide cleavage site is marked with an arrow, human homologues to bovine peptide sequences are underlined and the I-domain is boxed. Metal ion binding sites are indicated with a broken underline, potential N-glycosylation sites are indicated by an asterisk and the putative transmembrane domain is double underlined. The normally conserved cytoplasmic sequence 25 is indicated by a dot and dashed broken underline.

Sequence analysis demonstrate that  $\alpha 10$  is a member of the integrin  $\alpha$ -subunit family.

30

#### Example 4

Identification of a clone containing a splice variant of  $\alpha 10$

One clone which was isolated from the human chondrocyte library (see Example 3) contained a sequence 35 that was identical to the sequence of  $\alpha 10$  integrin sub-unit except that the nucleotides between nt positions

2942 and 3055 were deleted. The splice variant of  $\alpha 10$  was verified in PCR experiment using primers flanking the splice region (see figure 14).

5 Example 5

Identification of  $\alpha 10$  integrin subunit by Northern blot

Material and methods

Bovine chondrocyte mRNA was purified using a  
10 QuickPrep®Micro mRNA Purification Kit (Pharmacia Biotech,  
Uppsala, Sweden), separated on a 1% agarose-formaldehyde  
gel, transferred to nylon membranes and immobilised by UV  
crosslinking. cDNA-probes were 32P-labelled with Random  
Primed DNA Labeling Kit (Boehringer Mannheim). Filters  
15 were prehybridised for 2-4 hours at 42°C in 5x SSE,  
5x Denharts solution, 0.1 % SDS, 50 µg/ml salmon sperm  
DNA and 50% formamide and then hybridised over night  
at 42 °C with the same solution containing the specific  
probe (0.5-1 x 10<sup>6</sup> cpm/ml). Specifically bound cDNA-  
20 probes were analysed using the phosphoimager system  
(Fuji). Filters were stripped by washing in 0.1% SDS,  
for 1 hour at 80°C prior to re-probing. The  $\alpha 10$ -integrin  
cDNA-probe was isolated from the racel-containing plasmid  
using the restriction enzymes BamHI (GIBCO BRL) and NcoI  
25 (Boehringer Mannheim). The rat  $\beta 1$ -integrin cDNA probe was  
a kind gift from Staffan Johansson, Uppsala, Sweden.

Results

Northern blot analysis of mRNA from bovine chondro-  
cytes showed that a human  $\alpha 10$  cDNA-probe hybridised with  
30 a single mRNA of approximately 5.4 kb (Figure 7). As a  
comparison, a cDNA-probe corresponding to the integrin  
subunit  $\alpha 1$  was used. This cDNA-probe hybridised a mRNA-  
band of approximately 3.5 kb on the same filter. These  
results show that a cDNA-probe against  $\alpha 10$  can be used to  
35 identify the  $\alpha 10$  integrin subunit on the mRNA level.

**Example 6**

Preparation of antibodies against the integrin sub-unit  $\alpha 10$

A peptide corresponding to part of the  $\alpha 10$  cytoplasmic domain, Ckkipeekreekle (see figure 6) was synthesised and conjugated to keyhole limpet hemocyanin (KLH).  
5 Rabbits were immunised with the peptide-KLH conjugate to generate antiserum against the integrin subunit  $\alpha 10$ .  
Antibodies recognising  $\alpha 10$  were affinity purified on an  
10 peptide-coupled column (Innovagen AB).

**Example 7**

Immunoprecipitation of the integrin subunit  $\alpha 10$  from chondrocytes

**15 Material and methods**

Human chondrocytes were  $^{125}\text{I}$ -labelled, lysed with Triton X-100 and immunoprecipitated as earlier described (Holmvall et al, Exp Cell Res, 221, 496-503 (1995), Camper et al, JBC, 273, 20383-20389 (1998)). Triton X-100  
20 lysates of  $^{125}\text{I}$ -labeled human chondrocytes were immunoprecipitated with polyclonal antibodies against the integrin subunits  $\beta 1$ ,  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$  or  $\alpha 10$ . The immunoprecipitated proteins were separated by SDS-PAGE (4-12%) under non-reducing conditions and visualised using a phospho-  
25 imager. Triton X-100 lysates of human chondrocytes immunoprecipitated with  $\alpha 10$  or  $\beta 1$  were separated by SDS-PAGE (8%) under non-reducing conditions and analysed by Western blot using the polyclonal  $\beta 1$  antibody and chemiluminescent detection as described in Camper et al, JBC,  
30 273, 20383-20389 (1998).

**Results**

The polyclonal peptide antibody, raised against the cytoplasmic domain of  $\alpha 10$ , precipitated two protein bands with Mr of approximately 160 kD ( $\alpha 10$ ) and 125 kD ( $\beta 1$ )  
35 under reducing conditions. The  $\alpha 10$  associated  $\beta$ -chain migrated as the  $\beta 1$  integrin subunit (Figure 8a). To verify that the  $\alpha 10$  associated  $\beta$ -chain in chondrocytes

indeed is  $\beta 1$ , chondrocyte lysates were immunoprecipitated with antibodies against  $\alpha 10$  or  $\beta 1$  followed by Western blot using antibodies against the  $\beta 1$ -subunit (Figure 8b). These results clearly demonstrated that  $\alpha 10$  is a member 5 of the  $\beta 1$ -integrin family. However, the results do not exclude the possibility that  $\alpha 10$  can associate with other  $\beta$ -chains in other situations.

#### Example 8

10 Immunohistochemical staining of the integrin subunit  $\alpha 10$  in human and mouse cartilage

##### Material and methods

15 Frozen sections of adult cartilage (trochlear groove) obtained during surgery (provided by Anders Lindahl, Salgrenska Hospital, Gothenburg, Sweden and frozen sections from of 3 day old mouse limb were fixed and prepared for immunohistochemistry as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). Expression of  $\alpha 10$  integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a primary antibody (see Example 6) and a secondary antibody conjugated to peroxidase.

##### Results

25 Figures 9 show immunostaining of human adult articular cartilage.

The  $\alpha 10$ -antibody recognising the cytoplasmic domain of  $\alpha 10$  stained the chondrocytes in tissue sections of human articular cartilage (A). The staining was depleted when the antibody was preincubated with the  $\alpha 10$ - peptide 30 (B). A control antibody recognising the  $\alpha 9$  integrin subunit did not bind to the chondrocyte (C).

35 Figures 10 shows that the  $\alpha 10$  antibody stain the majority of chondrocytes in the growing bone anlage (a and b). The  $\alpha 10$  antibody also recognised cells in the ossification groove of Ranvier (b), especially the osteoblast in the bone bark which are lining the cartilage in the metaphys are highly positive for  $\alpha 10$ . The

cells in the ossification groove of Ranvier are believed to be important for the growth in diameter of the bone.

The integrin subunit  $\alpha 10$  is also highly expressed in perichondrium and periosteum. Cell in these tissues are

5 likely important in the repair of the cartilage tissue.

The described localisation of the integrin subunit  $\alpha 10$  suggest that this integrin is important for the function of the cartilage tissue.

10 Example 9

Immunohistochemical staining of the integrin subunit  $\alpha 10$  during mouse development

Material and methods

15 Frozen sections from mouse embryos (13.5 days) were investigated for expression of  $\alpha 10$  by immunohistochemistry as described in Camper et al, JBC, 273, 20383-20389 (1998). Expression of  $\alpha 10$  integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a primary antibody (see Example 6) and a secondary antibody conjugated to peroxidase. The embryo sections were also investigated for expression of integrin subunit  $\alpha 1$  (monoclonal antibody from Pharmingen) and collagen type II (monoclonal antibody, kind gift from Dr John Mo, Lund University, Sweden).

20 25 Results

Figure 11 show that  $\alpha 10$  integrin subunit is unregulated in the limb when the mesenchymal cells undergo condensation to form cartilage (a). Especially the edge of the newly formed cartilage has high expression of  $\alpha 10$ .

30 The formation of cartilage is verified by the high expression of the cartilage specific collage type II (b). The control antibody against  $\alpha 1$  integrin subunit showed only weak expression on the cartilage (c). In other experiments expression of  $\alpha 10$  was found in all cartilage containing tissues in the 3 day old mouse including limbs, ribs and vertebrae. The upregulation of  $\alpha 10$  during formation of cartilage suggest that this integrin subunit is

important both in the development of cartilage and bone and in the repair of damaged cartilage tissue.

#### Example 10

5 mRNA expression of  $\alpha 10$  in tissues other than articular cartilage

##### Material and methods

Expression of  $\alpha 10$  integrin subunit was examined on the mRNA level in different human tissues. A Northern blot with immobilised mRNA from the listed tissues in Figure 12 was hybridised with an  $\alpha 10$  integrin cDNA probe isolated from the race 1-containing plasmid using the restriction enzymes *Bam*H1 and *Nco*I. The degree of hybridisation was analysed using a phospho imager. The following symbols denote mRNA level in increasing order: -, +, ++, +++, ++++.

##### Results

Analysis of the hybridised mRNA showed that  $\alpha 10$  was expressed in aorta, trachea, spinal cord, heart, lung, and kidney (Figure 12). All other tissues appeared negative for  $\alpha 10$  expression. These results point to a restricted distribution of the  $\alpha 10$  integrin subunit.

#### Example 11

25 Immunohistochemical staining of  $\alpha 10$  in fascia around tendon and skeletal muscle and in tendon structures in heart valves.

##### Materials and methods

30 Frozen sections of adult cartilage (trochlear groove) obtained during surgery (provided by Anders Lindahl, Salgrenska Hospital, Gothenburg, Sweden and frozen sections from of 3 day old mouse limb were fixed and prepared for immunohistochemistry as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). Expression of  $\alpha 10$  integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a pri-

mary antibody (see Example 6) and a secondary antibody conjugated to peroxidase.

#### Results

As shown in figures 13 expression of  $\alpha 10$  was found  
5 in the fascia surrounding tendon (a) and skeletal muscle  
(b) and in the tendon structures in the heart valves (c).  
This localisation suggest that  $\alpha 10$  can bind to other  
matrix molecules in addition to the cartilage specific  
collagen type II. The localisation of the integrin  $\alpha 10$  on  
10 the surface of tendons indicate that  $\alpha 10$  can be involved  
in unwanted adhesion that often occurs between tendon/  
ligaments and the surrounding tissue after infection,  
injury or after surgery.

#### 15 Example 12

mRNA expression of  $\alpha 10$  integrin subunit in chondrocytes, endothelial cells and fibroblasts.

#### Material and methods

Isolation of mRNA, synthesis of cDNA and PCR amplification was done as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)).

#### Results

Figure 14 shows PCR amplification of  $\alpha 10$  cDNA from human articular chondrocytes (lanes A6 and B1), human umbilical vein endothelial cells (lane A2), human fibroblasts (lane A4) and rat tendon (Fig 14b, lane B2). Lanes 1, 3, and 5 in figure 14 A show amplified fragments corresponding to the integrin subunit  $\alpha 2$  in endothelial cells, fibroblasts and chondrocytes, respectively. cDNA-primers corresponding to the  $\alpha 10$  sequence positions nt 2919-2943 (forward) and nt 3554-3578 (reverse) (see Figure 6) were used to amplify  $\alpha 10$  cDNA from the different cells. The figure shows that  $\alpha 10$  was amplified in all three cell types. Two fragments of  $\alpha 10$  was amplified which represent the intact form of  $\alpha 10$  (larger fragment) and a splice variant (smaller fragment). The larger frag-

ment was dominating in chondrocytes while the smaller fragment was more pronounced in tendon (B2).

Example 13

5 Construction of  $\alpha 10$  mammalian expression vector.

The full length protein coding sequence of  $\alpha 10$  (combined from 3 clones, see figure 6) was inserted into the mammalian expression vector, pcDNA3.1/Zeo (Invitrogen). The vector contains SV40 promoter and Zeosin selection sequence. The  $\alpha 10$  containing expression vector was transfected into cells that express the  $\beta 1$ -integrin subunit but lack expression of the  $\alpha 10$  subunit. Expression of the  $\alpha 10$  integrin subunit on the cell surface can be analysed by immunoprecipitation and/or flow cytometry using antibodies specific for  $\alpha 10$ . The ligand binding capacity and the function of the inserted  $\alpha 10$  integrin subunit can be demonstrated in cell adhesion experiment and in signalling experiments.

20 Example 14

Construction of mammalian expression vector containing a splice variant of  $\alpha 10$ .

The full length protein coding sequence of the splice variant of  $\alpha 10$  (nt 2942-nt3055 deleted) was 25 inserted into the mammalian expression vector pcDNA3 (see Example 13). Expression and function of the splice variant can be analysed as described in example 13 and compared with the intact  $\alpha 10$  integrin subunit.

30 Example 15

Partial isolation and characterisation of the  $\alpha 10$  integrin genomic DNA

Material and methods

Human  $\alpha 10$  cDNA, isolated from the racel-containing 35 plasmid using the restriction enzymes *Bam*HI (GIBCO BRL) and *Nco*I (Boehringer Mannheim), was  $^{32}P$ -labelled and used as a probe for screening of a mouse 129 cosmid library

(provided by Reinhard Fässler, Lund University). Positive clones were isolated and subcloned. Selected plasmids were purified and sequenced as described earlier (Camper et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and 5 internal specific primers. Primers corresponding to mouse genomic DNA were then constructed and used in PCR to amplify and identify the genomic sequence of  $\alpha 10$  from the cosmid clones.

#### Results

10 Figure 15 shows 7958 nt of the  $\alpha 10$  gene. This partial genomic DNA sequence of  $\alpha 10$  integrin contains 8 exons, and a Kozak sequence. The mouse genomic  $\alpha 10$  sequence was used to generate a targeting vector for knockout experiments.

15

#### Example 16

Upregulation of  $\alpha 10$  integrin subunit in chondrocytes cultured in alginate beads

#### Material and methods

20 Human chondrocytes cultured in monolayer for 2 weeks were detached with trypsin-EDTA and introduced into alginate beads. Chondrocytes cultured in alginate are known to preserve their phenotype while chondrocytes cultured in monolayer are dedifferentiated. After 11 days chondro-  
25 cytes cultured either in alginate or on monolayer were isolated and surface labelled with  $^{125}\text{I}$ . The  $\alpha 10$  integrin subunit was then immunoprecipitated with polyclonal antibodies recognising the cytoplasmic domain of  $\alpha 10$  (see Example 6 and Camper et al, JBC, 273, 20383-20389  
30 (1998)).

#### Results

As shown in figure 16 chondrocytes cultured in alginate beads (lanes 3 and 4) upregulated their protein expression of  $\alpha 10\beta 1$ . This was in contrast to chondrocytes 35 cultured in monolayer (lanes 1 and 2) which had a very low expression of  $\alpha 10\beta 1$ . Immunoprecipitation with ab control antibody is shown in lanes 1 and 3. It is known that

chondrocytes preserve their cartilage specific matrix production in alginate cultures but not in monolayer culture which point to that alginate preserve the phenotype of chondrocytes. These results support that  $\alpha 10$  integrin 5 subunit can be used as a marker for differentiated chondrocytes.

#### Example 17

10 Immunoprecipitation of the  $\alpha 10$  integrin subunit from human smooth muscle cells.

#### Material and methods

Human smooth muscle cells were isolated from human aorta. After one week in culture the cells were  $^{125}\text{I}$ -labelled, lysed and immunoprecipitated with antibodies 15 against the integrin subunit  $\beta 1$  (lane 1),  $\alpha 1$  (lane 2),  $\alpha 2$  (lane 3),  $\alpha 10$  (lane 4),  $\alpha 3$  (lane 5), control (lane 6) (Figure 17). The experiment was done as described in Example 7.

#### Results

20 The  $\alpha 10$  antibody precipitated two bands from the smooth muscle cells corresponding to the  $\alpha 10$  and the  $\beta 1$  integrin subunit (Fig. 17).

#### Example 18

25 Construction of bacterial expression vector containing sequence for  $\alpha 10$  splice region.

A plasmid for intracellular expression in *E. coli* of the alternatively spliced region (amino acid pos. 952-986, SEQ. ID 1) was constructed as described. The 30 alternatively spliced region were back-translated using the *E. coli* high frequency codon table, creating a cDNA sequence of 96% identity with the original sequence (SEQ. ID 1 nucleotide pos 2940-3044). Using sequence overlap extension (Horton et al., *Biotechniques* 8:528, 1990) 35 primer  $\alpha 10\text{pfor}$  (tab. I) and  $\alpha 10\text{prev}$  (tab. I) was used to generate a double stranded fragment encoding the  $\alpha 10$  amino acid sequence. This fragment was used as a PCR

template with primers  $\alpha 10pfor2$  (tab. I) and  $\alpha 10prev2$  (tab. I) in order to generate restriction enzyme site for sub-cloning in a pET vector containing the Z-domain of staphylococcal protein A, creating a fusion of the  $\alpha 10$  spliced region with the amino terminal of the Z-domain with tronbin cleavage site residing in-between. The fragment generated in the second PCR reaction is shown (SEQ ID No. 3) also indicating the unique restriction enzymes used for sub-cloning in the expression vector.

10

Table I

$\alpha 10pfor$	5' - GTTCAAGAACCTGGGTTGCTACGTTGTTCCGGTCTGATCATCTCCGC TCTGCTGCCGGCTGT-3'
$\alpha 10pfor2$	5' -GGGGCATAATGGTTCAAGAACCTGGGTTGCTACGTTG-3'
$\alpha 10prev$	5' - GATAAACCTGGGACAAGCTTAGGAAGTAGTTACCAACCCTGAGCAACAG CCGGCAGCAGAGCGGA-3'
$\alpha 10prev2$	5' - GGGGGGATCCCGCGCGACCAGGCCGCTGATAACCTGGGACAAGCTT AGGAAGT-3'

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) NUMBER OF SEQUENCES: 2

## (2) INFORMATION FOR SEQ ID NO. 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3884 base pairs
- (B) TYPE: nucleic acid and amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (E) ORGANISM: human
- (F) CELLCYPE: chondrocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

CAGGTCA GAA ACC GAT CAGG C AT GGA ACT CCC CT CGT C ACT CAC CT GT T CTT GCCC CTG  
 1 -----+-----+-----+-----+-----+-----+-----+ 60  
 GTCCAGTCTTGGCTAGTCGTACCTTGAGGGAAAGCAGTGAGTGGACAAGAACGGGAC

a	M E L P F V T H L F L P L -
a	GTGTT CCTGACAGGTCTCTGCTCCCCCTTAACCTGGATGAACATCACCCACGCC TATTC 61 -----+-----+-----+-----+-----+-----+-----+ 120 CAC AAGGACTGTCCAGAGACGAGGGGAAATTGGACCTACTTGTAGTGGTGCGGATAAG
a	V F L T G L C S P F N L D E H H P R L F -
a	CCAGGGCCACCAGAAGCTGAATTGGATA CAGTGTCTTACAACATGTTGGGGGTGGACAG 121 -----+-----+-----+-----+-----+-----+-----+ 180 GGTCCC GG TGCTT CGACTTAAACCTATGTCA CAGAATGTTGTACAACCCCCACCTGTC
a	P G P P E A E F G Y S V L Q H V G G G Q -
a	CGATGGATGCTGGTGGCGCCCTGGATGGCCTTCAGGCACCGGAGGGGGACGTT 181 -----+-----+-----+-----+-----+-----+-----+ 240 GCTACCTACGACCACCCCGGGGAC CCTACCCGGAA GTCCGCTGGCCTCCCCCTGCAA
a	R W M L V G A P W D G P S G D R R G D V -
a	TATCGCTGCCCTGTAGGGGGGCCACAATGCCCATGTGCCAAGGGCCACTTAGGTGAC 241 -----+-----+-----+-----+-----+-----+-----+ 300 ATAGCGACGGGACATCCCCCGGGTGTACGGGTACACGGTCCGGTGAATCCACTG
a	Y R C P V G G A H N A P C A K G H L G D -
a	TACCAACTGGGAAATTCTCATCTCATCCTGCTGTGAATATGCACCTGGGATGTCTCTGTTA 301 -----+-----+-----+-----+-----+-----+-----+ 360 ATGGTTGACCTTTAAGTAGAGTAGGACGACACTTATACGTGGACCCCTACAGAGACAAT
a	Y Q L G N S S H P A V N M H L G M S L L -
a	GAGACAGATGGTATGGGGATT CATGGCCTGTGCCCTCTGGTCTCGTGTGGC 361 -----+-----+-----+-----+-----+-----+-----+ 420 CTCTGTCTACCACTACCCCTAAGTACCGGACACGGGAGAGACCAGAGCACGAACACCG
a	E T D G D G F M A C A P L W S R A C G -

AGCTCTGTCTTCAGTTCTGGGATATGTGCCCGTGTGGATGCTTCATTCCAGCCTCAGGGAA  
 421 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 480  
 TCGAGACAGAAGTCAAGACCCATAACACGGGCACACCTACGAAGTAAGTCGGAGTCCT  
  
 a S S V F S S G I C A R V D A S F Q P Q G -  
  
 AGCCTGGCACCCACTGCCAACGCTGCCAACATACATGGATGTTGTCATTGTCTGGAT  
 481 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 540  
 TCGGACCCTGGGTGACGGGTTGCGACGGGTTGTATGTACCTACAACAGTAACAGAACCTA  
  
 a S L A P T A Q R C P T Y M D V V I V L D -  
  
 GGCTCCAACAGCATCTACCCCTGGTCTGAAGTTCAGACCTCCTACGAAGACTGGTAGGG  
 541 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 600  
 CCGAGGTTGTCGTAGATGGGGACCAGACTCAAGTCTGGAAGGATGCTCTGACCATCCC  
  
 a G S N S I Y P W S E V Q T F L R R L V G -  
  
 AAACCTGTTATTGACCCAGAACAGATAACAGGTGGGACTGGTACAGTATGGGGAGAGCCCT  
 601 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 660  
 TTGACAAATAACTGGGTCTTGCTATGTCCACCCCTGACCATGTCATACCCCTCTCGGGAA  
  
 a K L F I D P E Q I Q V G L V Q Y G E S P -  
  
 GTACATGAGTGGTCCCTGGGAGATTCGAACGAAGGAAGAAGTGGTGAGAGCAGCAAAG  
 661 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 720  
 CATGTACTCACCAGGGACCCCTCTAAAGGCTTGCTCCTCTCACCACTCTCGTCGTTTC  
  
 a V H E W S L G D F R T K E E V V R A A K -  
  
 AACCTCAGTCGGCGGGAGGGACGAGAAAACAAAGACTGCCAACGAATAATGGTGGCCTGC  
 721 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 780  
 TTGGAGTCAGCCGCCCTCCCTGCTCTTGTGTTCTGACGGGTCGTTATTACCACCGGACG  
  
 a N L S R R E G R E T K T A Q A I M V A C -  
  
 ACAGAAGGGTTCACTCAGTCCCAGTGGGGCCGACCCGAGGCTGCCAGGCTACTGGTGGTT  
 781 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 840  
 TGCTCTCCCAAGTCAGTCAGGGTACCCCCGGCTGGCTCCGACGGTCCGATGACCACCAA  
  
 a T E G F S Q S H G G R P E A A R L L V V -  
  
 GTCACTGATGGAGAGTCCCATGATGGAGAGGAGCTTCTGCCAGCACTAAAGGCTGTGAG  
 841 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 900  
 CAGTGAATACCTCTCAGGGTACTACCTCTCGAACGGACGTGATTTCCGGACACTC  
  
 a V T D G E S H D G E E L P A A L K A C E -  
  
 GCTGGAAGAGTGACACGCTATGGGATTGCAGTCCTGGTCACTACCTCCGGCGGAGCGA  
 901 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 960  
 CGACCTTCTCACTGTGCGATAACCTAACGTCAGGAACCAAGTGATGGAGGCCGCGTCGCT  
  
 a A G R V T R Y G I A V L G H Y L R R Q R -  
  
 GATCCCAGCTTTCTGAGAGAAATTAGAAACTATTGCCAGTGTGATCCAGATGAGCGATTG  
 961 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020  
 CTAGGGTCGAGAAAGGACTCTCTTAATCTTGATAACGGTCACTAGGTCTACTCGCTAAG  
  
 a D P S S F L R E I R T I A S D P D E R F -  
  
 TTCTTCATGTCACAGATGAGGCTGCTCTGACTGACATTGTGGATGCACTAGGAGATCGG  
 1021 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1080  
 AAGAAGTTACAGTGTCTACTCCGACGAGACTGACTGTAACACCTACGTGATCCTCTAGCC  
  
 a F F N V T D E A A L T D I V D A L G D R -

1081 ATTTTTGGCCTTGAAGGGTCCCATGCAGAAAACGAAAGCTCCTTGGGCTGGAAATGTCT  
1140 TAAAAACCGGAACCTCCCAGGGTACGTCTTTGCTTCGAGGAAACCCGACCTTACAGA  
a I F G L E G S H A E N E S S F G L E M S -  
1141 CAGATTGGTTCTCCACTCATCGGCTAAAGGATGGGATTCTTTGGGATGGTGGGGGCC  
1200 GTCTAACCAAAGAGGTGAGTAGCCGATTCTACCCCTAACGAAAAACCCCTACCACCCCCGG  
a Q I G F S T H R L K D G I L F G M V G A -  
1201 TATGACTGGGGAGGCTCTGTCTATGGCTTGAAGGAGGCCACCGCCTTCCCCCACGA  
1260 ATACTGACCCCTCCGAGACACGATAACGAACTCCTCCGGTGGCGGAAAGGGGGTGCT  
a Y D W G G S V L W L E G G H R L F P P R -  
1261 ATGGCACTGGAAGACGAGTTCCCCCTGCACTGCAGAACCATGCAGCCTACCTGGGTTAC  
1320 TACCGTGACCTCTGCTCAAGGGGGACGTGACGTCTTGGTACGTGGATGGACCCAATG  
a M A L E D E F P P A L Q N H A A A Y L G Y -  
1321 TCTGTTCTCCATGCTTTCGGGGTGGACGCCCTGTTCTCTCTGGGCTCCTCGA  
1380 AGACAAAGAAGGTACGAAACGCCACCTGCGGGGACAAAGAGAGACCCGAGGAGCT  
a S V S S M L L R G G R R L F L S G A P R -  
1381 TTAGACATCGAGGAAAGTCATGCCCTCCAGCTTAAGAAAGATGGGCTGTGAGGGTT  
1440 AAATCTGTAGCTCCTTTCACTAGCGGAAGGTGAATTCTTCTACCCCGACACTCCAA  
a F R H R G K V I A F Q L K K D G A V R V -  
1441 GCCCAGAGCCTCCAGGGGAGCAGATGGTTCATACTTGGCAGTGAGCTCTGCCATTG  
1500 CGGGTCTCGGAGGTCCCCCTCGTCAACCAAGTATGAAACCGTCACTCGAGACGGTAAC  
a A Q S L Q G E Q I G S Y F G S E L C P L -  
1501 GATACAGATAGGGATGGAACAACGTGATGTCTTACTTGTGGCTGCCCATGTTCTGGGA  
1560 CTATGTCTATCCCTACCTTGTGACTACAGAATGAAACACCGACGGGGTACAAGGACCT  
a D T D R D G T T D V L L V A A A P M F L G -  
1561 CCCCAGAACAGGAAACAGGACGTGTTATGTATCTGGTAGGCCAGCAGTCCTGCTG  
1620 GGGGTCTTGTCTTGTGCTGCACAAATACACATAGACCATCCGGTCGTAGGAACGAC  
a P Q N K E T G R V Y V Y L V G Q Q S L L -  
1621 ACCCTCCAAGGAACACTTCAGCCAGAACCCCCCAGGATGCTCGGTTGGCTTGCCATG  
1680 TGGGAGGTTCTTGTGAAGTCGGCTTGGGGGGCCTACGAGCCAAACCGAAACGGTAC  
a T L Q G T L Q P E P P Q D A R F G F A M -  
1681 GGAGCTCTTCTGATCTGAACCAAGATGGTTTGCTGATGTGGCTGTGGGGCGCCTCTG  
1740 CCTCGAGAAGGACTAGACTTGGTCTACCAAAACGACTACACCGACACCCCCGGAGAC  
a G A L P D L N Q D G F A D V A V G A P L -

1741 GAAGATGGGACCAGGGAGCACTGTACCTGTACCATGGAACCCAGAGTGGAGTCAGGCC  
 CTTCTACCCGTGGTCCCTCGTGACATGGACATGGTACCTTGGTCTCACCTCAGTCCGG 1800  
 a E D G H Q G A L Y L Y H G T Q S G V R P -  
 1801 CATCCTGCCAGAGGATTGCTGCTGCCATGCCACATGCCCTCAGCTACTTGGCGA  
 GTAGGACGGGTCTCCTAACGACGACGGAGGTACGGTGTACGGAGTCGATGAAACCGGCT 1860  
 a H P A Q R I A A A S M P H A L S Y F G R -  
 1861 AGTGTGGATGGTCGGCTAGATCTGGATGGAGATGATCTGGTCATGTGGCTGTGGGTGCC  
 TCACACCTACCAGCCGATCTAGACCTACCTACTAGACCAGTACACCGACACCCACGG 1920  
 a S V D G R L D L D G D D L V D V A V G A -  
 1921 CAGGGGGCAGCCATCCTGCTCAGCTCCGGCCATTGTCCATCTGACCCCACACTGGAG  
 GTCCCCCGTCCGGTAGGACCGAGTCGAGGGCCGGTAACAGGTAGACTGGGTAGTGACCTC 1980  
 a Q G A A I L L S S R P I V H L T P S L E -  
 1981 GTGACCCCACAGGCCATCAGTGTGGTCAGAGGGACTGTAGGCAGGCGAGGCCAAGAACCA  
 CACTGGGTGTCCGGTAGTCACACCAAGTCTCCCTGACATCCGCCGCTCCGGTTCTCGT 2040  
 a V T P Q A I S V V Q R D C R R R G Q E A -  
 2041 GTCTGTCTGACTGCAGCCCTTGCTTCCAAGTGCACCTCCGTACTCCTGGTCGCTGGAT  
 CAGACAGACTGACGTCGGAAACGAAGGTTACTGGAGGGCATGAGGACCGACCGACCTA 2100  
 a V C L T A A L C F Q V T S R T P G R W D -  
 2101 CACCAATTCTACATGAGGTTACCGCATCACTGGATGAATGGACTGCTGGGCACGTGCA  
 GTGGTTAAGATGTACTCCAAGTGGCTAGTGACCTACTTACCTGACGACCCGTGCACGT 2160  
 a H Q F Y M R F T A S L D E W T A G A R A -  
 2161 GCATTTGATGGCTCTGGCCAGAGGTTGTCGGCTCGGAGGCTCCGGCTCAGTGTGGGAAT  
 CGTAAACTACCGAGACCGGCTCCAACAGGGGAGCCCTCCGAGGCCGAGTCACACCCCTA 2220  
 a A F D G S G Q R L S P R R L R L S V G N -  
 2221 GTCACTTGTGAGCAGCTACACTCCATGTGCTGGATACTCAGATTACCTCCGGCCAGTG  
 CAGTGAACACTCGTCGATGTGAAGGTACACGACCTATGTAGTCTAATGGAGGCCGGTCAC 2280  
 a V T C E Q L H F H V L D T S D Y L R P V -  
 2281 GCCTTGACTGTGACCTTGGCTTGACAATACTACAAAGCCAGGGCCTGTGCTGAATGAG  
 CGGAACACTGGAAACGGAACCTGTTATGATGTTCGGTCCCGACACGACTTACTC 2340  
 a A L T V T F A L D N T T K P G P V L N E -  
 2341 GGCTCACCCACCTCTATAACAAAAGCTGGTCCCTCTCAAAGGATTGTGGCCCTGACAAT  
 CCGAGTGGGTGGAGATATGTTCGACCAGGGAAAGAGTTCTAACACCGGGACTGTTA 2400  
 a G S P T S I Q K L V P F S K D C G P D N -

2401 GAATGTGTACAGACCTGGTCTTCAGTGAATATGGACATCAGAGGCTCCAGGAAGGCC  
 CTTACACAGTGTCTGGACCACGAAGTTCACTTATACTGTAGTCTCGAGGTCTTCGG  
 a E C V T D L V L Q V N M D I R G S R K A -  
 2461 CCATTTGTGGTTCGAGGTGGCCGGCGAAAGTGCCTGGTATCTACAACCTGGAGAACAGA  
 GGTAAACACCAAGCTCCACCGGCCCTTCACGACCATAGATGTTGAGACCTCTTGCT  
 a P F V V R G G R R K V L V S T T L E N R -  
 2521 AAGGAAAATGCTTACAATACAGAGCCTGAGTATCATCTTCTCTAGAAACCTCCACCTGGCC  
 TTCCCTTTACGAATGTTATGCTCGGACTCATAGTAGAAGAGATCTTGAGGTGGACCGG  
 a K E N A Y N T S L S I I F S R N L H L A -  
 2581 AGTCTCACTCCTCAGAGAGAGGCCAATAAGGTGGAATGTGCCGCCCTCTGCTCAT  
 TCAGAGTGAGGAGTCTCTCTCGGGTTATTCCACCTTACACGGCGGGGAAGACGAGTA  
 a S L T P Q R E S P I K V E C A A P S A H -  
 2641 GCCCGGCTCTGCAGTGTGGGGCATCCTGTCTTCCAGACTGGAGCCAAGGTGACCTTCTG  
 CGGGCCGAGACGTCACACCCCGTAGGACAGAAGGTCTGACCTCGGTCCACTGGAAAGAC  
 a A R L C S V G H P V F Q T G A K V T F L -  
 2701 CTAGAGTTGAGTTAGCTGCTCCTCTCCTGAGCCAGGTCTTGGGAAGCTGACTGCC  
 GATCTCAAACCTCAAATCGACGAGGAGAGGACTCGGTCCAGAAACCCCTCGACTGACGG  
 a L E F E F S C S S L L S Q V F G K L T A -  
 2761 AGCAGTGACAGCCTGGAGAGAAATGGCACCCCTCAAGAAAACACAGCCCAGACCTCAGCC  
 TCGTCACTGTCGGACCTCTCTTACCGTGGGAAGTTCTTGTGTCGGCTGGAGTCGG  
 a S S D S L E R N G T L Q E N T A Q T S A -  
 2821 TACATCCAATATGAGCCCCACCTCCTGTTCTAGTGAATCTACCCCTGCACCGCTATGAG  
 ATGTAGGTTATACTGGGGTGGAGGACAAGAGATCACTCAGATGGACGTGGCGATACTC  
 a Y I Q Y E P H L L F S S E S T L H R Y E -  
 2881 GTTCACCCATATGGGACCCCTCCAGTGGTCTGGCCAGAATTCAAACCACTCTCAGG  
 CAAGTGGGTATACCTGGGAGGGTCACCCAGGACCGGGCTTAAGTTGGTGGAGTCGG  
 a V H P Y G T L P V G P G P E F K T T L R -  
 2941 GTTCAGAACCTAGGCTGCTATGTGGTCAAGTGGCTCATCATCTCAGCCCTCCAGCT  
 CAAGTCTGGATCCGACGATAACCCAGTCACCGGAGTAGTAGAGTCGGGAGGAAGGTGCA  
 a V Q N L G C Y V V S G L I I S A L L P A -  
 3001 GTGGCCCATGGGGCAATTACTCCTATCACTGTCAGTCAGTCAACTAACATGCAAGC  
 CACCGGGTACCCCGTTAATGAAGGATAGTGACAGAGTCAGTAGTGATTGTTACGTTG  
 a V A H G G N Y F L S L S Q V I T N N A S -

41

3061 TGCATAGTGCAGAACCTGACTGAACCCCCAGGCCACCTGTGCATCCAGAGGGAGCTTCAA  
 3120 ACGTATCACGTCTGGACTGACTTGGGGTCCGGGTGGACACGTAGGTCTCCTCGAAGTT 3120

a C I V Q N L T E P P G P P V H P E E L Q -

3121 CACACAAACAGACTGAATGGGAGCAATACTCAGTGTCAAGTGGTGGAGGTGCCACCTGGG  
 3180 GTGTGTTGACTTACCCCTCGTTATGAGTCACAGTCCACCACTCCACGGTGGAACCC 3180

a H T N R L N G S N T Q C Q V V R C H L G -

3181 CAGCTGGCAAAGGGGACTGAGGTCTCTGTTGGACTATTGAGGCTGGTCACAATGAATT  
 3240 GTGCACCGTTCCTCCCTGACTCCAGAGACAACCTGATAACTCCGACCAAGTGTACTAAA 3240

a Q L A K G T E V S V G L L R L V H N E F -

3241 TTCCGAAGAGCCAAGTTCAAGTCCCTGACGGTGGTCAGCACCTTGAGCTGGAACCGAA  
 3300 AAGGCTTCTCGGTTCAAGTTCAAGGGACTGCCACCAGTCGTGGAAACTCGACCCCTGGCTT 3300

a F R R A K F K S L T V V S T F E L G T E -

3301 GAGGGCAGTGTCCCTACAGCTGACTGAAGCCTCCCCTGGAGTGAGAGCCTTGGAGGTG  
 3360 CTCCCGTCACAGGATGTCGACTGACTTCGGAGGGCACCTCACTCGGAGAACCTCCAC 3360

a E G S V L Q L T E A S R W S E S L L E V -

3361 GTTCAGACCCGGCCTATCCTCATCTCCCTGTGGATCCTCATAGGCAGTGTCTGGAGGG  
 3420 CAAGTCTGGGCCGGATAGGAGTAGAGGGACACCTAGGAGTATCCGTACAGGACCCCTCCC 3420

a V Q T R P I L I S L W I L I G S V L G G -

3421 TTGCTCCTGCTTGCTCTCCTGTCTTGTGGCTGTGGAAAGCTTGGCTTCTTGCCCATAAG  
 3480 AACGAGGACGAAACGAGAGGAACAGAAAGACGGACACCTTCGAACCGAAGAACGGTATTG 3480

a L L L A L L V F C L W K L G F F A H K -

3481 AAAATCCCTGAGGAAGAAAAAGAGAAGAGAAGTGGAGCAATGAATGTAGAATAAGGGT  
 3540 TTTAGGGACTCCTCTTTCTCTCTCAACCTCGTTACTTACATCTTATTCCCA 3540

a K I P E E E K R E E K L E Q

3541 CTAGAAAGTCCTCCCTGGCAGCTTCTCAAGAGACTTGCATAAAAGCAGAGGTTGGGG  
 3600 GATCTTCAGGAGGGACCGTCGAAAGAAGTCTCTGAACGTATTTCGTCTCCAAACCCC 3600

3601 GCTCAGATGGGACAAGAACGCCCTCTGGACTATCTCCCCAGACCAGCAGCCTGACTTGA  
 3660 CGAGTCTACCCCTGTTCTCGGCGGAGACCTGATAGAGGGGTCTGGTCGTGGACTGAAC 3660

3661 CTTTGAGTCCTAGGGATGCTGCTGGTAGAGATGAGGCTTACCTCAGACAAGAACAGC  
 3720 GAAAACTCAGGATCCCTACGACGACCGATCTACTCCGAAATGGAGTCTGTTCTCG 3720

3721 TGGCACCAAAACTAGCCATGCTCCCACCCCTCTGCTTCCCTCCTCGT GATCCTGGTTC  
-----+-----+-----+-----+-----+-----+-----+ 3780  
ACCGTGGTTTGATCGGTACGAGGGTGGGAGACGAAGGGAGGGAGCACTAGGACCAAG

3781 CATAGCCAACACTGGGGCTTTGTTGGGTCTTTATCCCCAGGAATCAATAATT  
-----+-----+-----+-----+-----+-----+-----+ 3840  
GTATCGGTTGTGACCCCGAAAACAAACCCAGGAAAATAGGGTCCTTAGTTATTAAAAA

3841 TTGCCTAGGAAAAAAAAAGCGGCCGCGAATTGATATCAAGCT  
-----+-----+-----+-----+-----+-----+-----+ 3884  
AACGGATCCTTTTTTCGCCGGCGCTTAAGCTATAGTCGA

## (2) INFORMATION FOR SEQ ID NO. 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3779 base pairs
- (B) TYPE: nucleic acid and amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (E)

## (i) MOLECULAR TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (B) CELLCYPE: chondrocyte

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

CAGGTCAAGAACCGATCAGGCATGGAACCTCCCTTCGTCACTCACCTGTTCTGCCCTG  
 1 -----+-----+-----+-----+-----+-----+ 60  
 GTCCAGTCTTGCTAGTCCGTACCTTGAGGGAAAGCAGTGAGTGGACAAGAACGGGAC

	M	E	L	P	F	V	T	H	L	F	L	P	L	-
--	---	---	---	---	---	---	---	---	---	---	---	---	---	---

GTGTTCCGTACAGGTCTCTGCTCCCCCTTAACCTGGATGAACATCACCCACGCCATTTC  
 61 -----+-----+-----+-----+-----+-----+-----+-----+ 120  
 CACAAGGACTGTCCAGAGACGAGGGGAAATTGGACCTACTTGTAGTGGTGCGGATAAG

a	V	F	L	T	G	L	C	S	P	F	N	L	D	E	H	H	P	R	L	F	-
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

CCAGGGCCACCAGAACGCTGAATTGGATACAGTGTCTTACAACATGTTGGGGTGGACAG  
 121 -----+-----+-----+-----+-----+-----+-----+-----+ 180  
 GGTCCCGGTGGCTTCGACTTAAACCTATGTACAGAAATGTTGTACAACCCCCCACCTGTC

a	P	G	P	P	E	A	E	F	G	Y	S	V	L	Q	H	V	G	G	G	Q	-
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

CGATGGATGCTGGTGGCGCCCCCTGGGATGGGCCTCAGGGACCGGAGGGGGACGTT  
 181 -----+-----+-----+-----+-----+-----+-----+-----+ 240  
 GCTACCTACGACCACCCGGGGGACCTACCCGGAAGTCCGCTGGCCTCCCCCTGCAA

a	R	W	M	L	V	G	A	P	W	D	G	P	S	G	D	R	R	G	D	V	-
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

TATCGCTGCCCTGTAGGGGGCCCACAATGCCCATGTGCCAAGGGCCACTTAGGTGAC  
 241 -----+-----+-----+-----+-----+-----+-----+-----+ 300  
 ATAGCGACGGGACATCCCCCCGGGTGTTACGGGTACCGGTTCCGGTGAATCCACTG

a	Y	R	C	P	V	G	G	A	H	N	A	P	C	A	K	G	H	L	G	D	-
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

TACCAACTGGAAATTCTCATCTCATCCTGCTGTGAATATGCACCTGGGATGTCTGTGTTA  
 301 -----+-----+-----+-----+-----+-----+-----+-----+ 360  
 ATGGTTGACCTTTAACGTAGAGTAGGACGACACTTACACGTGGACCCCTACAGAGACAAT

a	Y	Q	L	G	N	S	S	H	P	A	V	N	M	H	L	G	M	S	L	L	-
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

GAGACAGATGGTGTGGGATTCATGGCCTGTGCCCTCTCTGGTCTCGTGTGGC  
 361 -----+-----+-----+-----+-----+-----+-----+-----+ 420  
 CTCTGTCTACCACTACCCCTAACGTACCGGACACGGGAGAGACCAGAGCACGAACACCG

a	E	T	D	G	D	G	G	F	M	A	C	A	P	L	W	S	R	A	C	G	-
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

AGCTCTGTCTTCAGTTCTGGGATATGTGCCCGTGTGGATGCTTCATTCCAGCCTCAGGGA  
 421 -----+-----+-----+-----+-----+-----+-----+-----+ 480  
 TCGAGACAGAAGTCAAGACCCCTATACACGGCACACCTACGAAGTAAGGTCGGAGTCCT

44

a S S V F S S G I C A R V D A S F Q P Q G -  
 AGCCTGGCACCCACTGCCAACGCTGCCAACATACTGGATGTTGTCATTGTCTTGGAT  
 481 -----+-----+-----+-----+-----+-----+-----+ 540  
 TCGGACCGTGGGTGACGGGTTGCGACGGGTTGTATGTACCTACAACAGTAACAGAACCTA

a S L A P T A Q R C P T Y M D V V I V L D -  
 GGCTCCAACACCATCTACCCCTGGTCTGAAGTTCAAGACCTCCTACGAAGACTGGTAGGG  
 541 -----+-----+-----+-----+-----+-----+-----+ 600  
 CCGAGGTTGTCGTAGATGGGACCAGACTCAAGTCTGGAAGGATGCTCTGACCATCCC

a G S N S I Y P W S E V Q T F L R R L V G -  
 AAACCTGTTTATTGACCCAGAACAGATAACAGGGACTGGTACAGTATGGGGAGAGCCCT  
 601 -----+-----+-----+-----+-----+-----+-----+ 660  
 TTTGACAAATAACTGGGTCTTGTCTATGTCCACCTGACCATGTACACCCCTCTCGGGA

a K L F I D P E Q I Q V G L V Q Y G E S P -  
 GTACATGAGTGGTCCCTGGGAGATTCCGAACGAAGGAAGAAGTGGTGAGAGCAGCAAAG  
 661 -----+-----+-----+-----+-----+-----+-----+ 720  
 CATGTACTCACCAAGGGACCCCTAAAGGCTTGCTTCCTTCTCACCACTCTCGTCGTTTC

a V H E W S L G D F R T K E E V V R A A K -  
 AACCTCAGTCGGCGGGAGGGACGAGAACAAAGACTGCCAACGAAATAATGGTGGCCTGC  
 721 -----+-----+-----+-----+-----+-----+-----+ 780  
 TTGGAGTCAGCGCCCTCCCTGCTTTGTTCTGACGGGTTGTTATTACCAACCGGACG

a N L S R R E G R E T K T A Q A I M V A C -  
 ACAGAAGGGTTCAAGTCAGTCCCAGTGGGGCCGACCCGAGGCTGCCAGGCTACTGGTGGTT  
 781 -----+-----+-----+-----+-----+-----+-----+ 840  
 TGTCTTCCCAAGTCAGTCAGGTACCCCCGGCTGGGCTCCGACGGTCCGATGACCACCAA

a T E G F S Q S H G G R P E A A R L L V V -  
 GTCACTGATGGAGAGTCCCATGATGGAGAGGAGCTTCCTGCAGCACTAAAGGCCTGTGAG  
 841 -----+-----+-----+-----+-----+-----+-----+ 900  
 CAGTGACTACCTCTCAGGGTACTACCTCTCGAAGGACGTGCGTGAATTCCGGACACTC

a V T D G E S H D G E E L P A A L K A C E -  
 GCTGGAAGAGTGACACGCTATGGGATTGCAGTCCTGGTCACTACCTCCGGCGAGCGA  
 901 -----+-----+-----+-----+-----+-----+-----+ 960  
 CGACCTCTCACTGTGCGATAACCTAACGTCAAGAACAGTGATGGAGGCCGCGTCGCT

a A G R V T R Y G I A V L G H Y L R R Q R -  
 GATCCCAGCTTTCTGAGAGAAATTAGAACTATTGCCAGTGATCCAGATGAGCGATTG  
 961 -----+-----+-----+-----+-----+-----+-----+ 1020  
 CTAGGGTCGAGAAAGGACTCTCTTAATCTGATAACGGTCACTAGGTCTACTCGCTAAG

a D P S S F L R E I R T I A S D P D E R F -  
 TTCTCAATGTCACAGATGAGGCTGCTCTGACTGACATTGTGGATGCACTAGGAGATCGG  
 1021 -----+-----+-----+-----+-----+-----+-----+ 1080  
 AAGAAGTTACAGTGTCTACTCCGACGAGACTGACTGTAACACCTACGTGATCCTCTAGCC

a F F N V T D E A A L T D I V D A L G D R -  
 ATTTTGCCCTTGAAGGGTCCCAGCAGAAACGAAAGCTCCTTGGGCTGGAAATGTCT  
 1081 -----+-----+-----+-----+-----+-----+-----+ 1140  
 TAAAAACCGGAACCTCCCAGGGTACGTCTTGCTTCGAGGAAACCCGACCTTACAGA

45

a	I F G L E G S H A E N E S S F G L E M S -
	CAGATTGGTTCTCCACTCATCGGCTAAAGGATGGGATTCTTTGGGATGGTGGGGGCC
1141	-----+-----+-----+-----+-----+-----+ 1200 GTCTAACCAAAGAGGTGAGTAGCCATTCTACCCCTAACGAAAACCTACCACCCCCGG
a	Q I G F S T H R L K D G I L F G M V G A -
	TATGACTGGGGAGGCCTCTGTGCTATGGCTTGAAGGGAGGCCACCGCCTTTCCCCCACGA
1201	-----+-----+-----+-----+-----+-----+ 1260 ATACTGACCCCTCCGAGACACGATACCGAACCTCCCTCGGTGGCGAAAAGGGGGTGCT
a	Y D W G G S V L W L E G G H R L F P P R -
	ATGGCACTGGAAGACGAGTCACCGACTGCAGAACCATGCAGCCTACCTGGGTTAC
1261	-----+-----+-----+-----+-----+-----+ 1320 TACCGTGAACCTTCTGCTCAAGGGGGACGTGACGTCTGGTACGTCGGATGGACCCAATG
a	M A L E D E F P P A L Q N H A A A Y L G Y -
	TCTGTTCTTCCATGCTTTCGGGGTGGACGCCCTGTTCTCTGGGGCTCCTCGA
1321	-----+-----+-----+-----+-----+-----+ 1380 AGACAAAGAAGGTACGAAAACGCCACCTGCGCGGACAAAGAGAGACCCGAGGAGCT
a	S V S S M L L R G G R R L F L S G A P R -
	TTTAGACATCGAGGAAAAGTCATCGCCTTCAGCTTAAGAAAGATGGGGCTGTGAGGGTT
1381	-----+-----+-----+-----+-----+-----+ 1440 AAATCTGTAGCTCCTTTCAGTAGCGGAAGGTCGAATTCTTCTACCCGACACTCCAA
a	F R H R G K V I A F Q L K K D G A V R V -
	GCCCAGAGCCTCCAGGGGAGCAGATTGGTCATACTTGGCAGTGAGCTCTGCCATTG
1441	-----+-----+-----+-----+-----+-----+ 1500 CGGGTCTCGGAGGTCCCCCTCGTCAACCAAGTATGAAACCGTCACTCGAGACGGTAAC
a	A Q S L Q G E Q I G S Y F G S E L C P L -
	GATACAGATAGGGATGGAACAACGTGATGTCTTACTTGTGGCTGCCCCATGTTCTGGGA
1501	-----+-----+-----+-----+-----+-----+ 1560 CTATGTCTATCCCTACCTTGTGACTACAGAATGAACACCGACGGGGTACAAGGACCT
a	D T D R D G T T D V L L V A A P M F L G -
	CCCCAGAACAGGAAACAGGACGTGTTATGTGATCTGGTAGGCCAGCAGTCCTGCTG
1561	-----+-----+-----+-----+-----+-----+ 1620 GGGGTCTTGTTCCTTGTCCCTGCACAAATACACATAGACCATCCGGTCGTCAAGGAACGAC
a	P Q N K E T G R V Y V Y L V G Q Q S L L -
	ACCCCTCCAAGGAACACTTCAGCCAGAACCCCCCAGGATGCTCGGTTGGCTTGCATG
1621	-----+-----+-----+-----+-----+-----+ 1680 TGGGAGGTTCTTGTGAAGTCGGCTTGGGGGCTACGAGCAAACCGAAACGGTAC
a	T L Q G T L Q P E P P Q D A R F G F A M -
	GGAGCTCTCCTGATCTGAACCAAGATGGTTTGCTGATGTGGCTGTGGGGCGCTCTG
1681	-----+-----+-----+-----+-----+-----+ 1740 CCTCGAGAAGGACTAGACTTGGTTCTACCAAACGACTACACCGACACCCCCGGAGAC
a	G A L P D L N Q D G F A D V A V G A P L -
	GAAGATGGGCACCAAGGGAGCACTGTACCTGTACCATGGAACCCAGAGTGGAGTCAGGCC
1741	-----+-----+-----+-----+-----+-----+ 1800 CTTCTACCCGGTGGTCCCTCGTGACATGGACATGGTACCTTGGTCTCACCTCAGTCGGGG

46

a E D G H Q G A L Y L Y H G T Q S G V R P -  
 1801 CATCCTGCCAGAGGATTGCTGCTGCCTCCATGCCACATGCCCTCAGCTACTTGGCCGA  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1860  
 GTAGGACGGGTCTCTAACGACGACGGAGGTACGGTGTACGGAGTCGATGAAACCGGCT

a H P A Q R I A A A S M P H A L S Y F G R -  
 1861 AGTGTGGATGGTCGGCTAGATCTGGATGGAGATGATCTGGTCATGTGGCTGTGGGTGCC  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1920  
 TCACACCTACCAGCCGATCTAGACCTACCTCTACTAGACCAGCTACACCGACACCCACGG

a S V D G R L D L D G D D L V D V A V G A -  
 1921 CAGGGGGCAGCCATCCTGCTCAGCTCCGGCCCATTGTCCATCTGACCCCACACTGGAG  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1980  
 GTCCCCCGTCGGTAGGACGAGTCGAGGGCCGGTAACAGGTAGACTGGGTAGTGACCTC

a Q G A A I L L S S R P I V H L T P S L E -  
 1981 GTGACCCACAGGCCATCAGTGTGGTTAGGGACTGTAGGCAGGCGAGGCCAAGAAGCA  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2040  
 CACTGGGGTGTCCGGTAGTCACACCAAGTCTCCCTGACATCCGCCCTCCGGTCTTCGT

a V T P Q A I S V V Q R D C R R R G Q E A -  
 2041 GTCTGTCTGACTGCAGCCCTTGCTTCCAAGTGAACCTCCCGTACTCCTGGTCGCTGGAT  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2100  
 CAGACAGACTGACGTGGAAACGAAGGTTCACTGGAGGGCATGAGGACCAGCGACCTA

a V C L T A A L C F Q V T S R T P G R W D -  
 2101 CACCAATTCTACATGAGGTTCACCGCATCACTGGATGAATGGACTGCTGGGCACGTGCA  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2160  
 GTGGTTAAGATGTACTCCAAGTGGCGTAGTGACCTACTTACCTGACGACCCGTGCACGT

a H Q F Y M R F T A S L D E W T A G A R A -  
 2161 GCATTTGATGGCTCTGCCAGAGGTTGCCCCCTGGAGGCTCCGGCTCAGTGTGGGAAT  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2220  
 CGTAAACTACCGAGACCGGTCTCAAACAGGGAGCCCTCGAGGCCAGTCACACCCCTTA

a A F D G S G Q R L S P R R L R L S V G N -  
 2221 GTCACTTGTGAGCAGCTACACTCCATGTGCTGGATACATCAGATTACCTCCGGCCAGTG  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2280  
 CAGTGAACACTCGTCGATGTGAAGGTACACGACCTATGTAGTCTAATGGAGGCCGGTCAC

a V T C E Q L H F H V L D T S D Y L R P V -  
 2281 GCCTTGACTGTGACCTTGGCTTGACAATACTACAAAGCCAGGGCTGTGCTGAATGAG  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2340  
 CGGAACTGACACTGGAAACGGAACCTGTTATGATGTTGGTCCGGACACGACTTACTC

a A L T V T F A L D N T T K P G P V L N E -  
 2341 GGCTCACCCACCTCTATACAAAGCTGGTCCCTCTCAAAGGATTGTGGCCCTGACAAT  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2400  
 CCGAGTGGGTGGAGATATGTTTCGACCAGGGAAAGAGTTCTAACACCGGGACTGTTA

a G S P T S I Q K L V P F S K D C G P D N -  
 2401 GAATGTGTCACAGACCTGGTGTCAAGTGAATATGGACATCAGAGGCTCCAGGAAGGCC  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2460  
 CTTACACAGTGTCTGGACCACGAAGTTCACTTACCTGTTAGTCTCCGAGGTCCCTCCGG

a E C V T D L V L Q V N M D I R G S R K A -  
 2461 CCATTGTTGGTCGAGGTGGCCGGCGAAAGTGCCTGGATCTACAACTCTGGAGAACAGA  
 2520 GGTAAACACCAAGCTCCACCGGCCCTTCACGACCATAGATGTTGAGACCTCTGTCT

a P F V V R G G R R K V L V S T T L E N R -  
 2521 AAGGAAAATGCTTACAATACGAGCCTGAGTATCATCTTCTCTAGAAACCTCCACCTGGCC  
 2580 TTCCCTTTACGAATGTTATGCTCGGACTCATAGTAGAAGAGATCTTGGAGGTGGACCGG

a K E N A Y N T S L S I I F S R N L H L A -  
 2581 AGTCTCACTCCTCAGAGAGAGGCCAATAAGGTGGAATGTGCCGCCCTCTGCTCAT  
 2640 TCAGAGTGAGGAGTCTCTCTCGGGTTATTCCACCTACACGGCGGGGAAGACGAGTA

a S L T P Q R E S P I K V E C A A P S A H -  
 2641 GCCCGGCTCTGCAGTGTGGGCATCCTGTCTCCAGACTGGAGCCAAGGTGACCTTCTG  
 2700 CGGGCCGAGACGTACACCCCCGTAGGACAGAAGGTCTGACCTCGGTTCCACTGGAAAGAC

a A R L C S V G H P V F Q T G A K V T F L -  
 2701 CTAGAGTTGAGTTAGCTGCTCCTCTCCCTGAGCCAGGTCTTGGGAAGCTGACTGCC  
 2760 GATCTCAAACCTCAAATCGACGAGGAGAGGACTCGGTCCAGAAACCCCTCGACTGACGG

a L E F E F S C S S L L S Q V F G K L T A -  
 2761 AGCAGTGACAGCCTGGAGAGAAATGGCACCCCTCAAGAAAACACAGCCCAGACCTCAGCC  
 2820 TCGTCACTGTCGGACCTCTCTTACCGTGGAAAGTCTTGTGTCGGTCTGGAGTCGG

a S S D S L E R N G T L Q E N T A Q T S A -  
 2821 TACATCCAATATGAGCCCCACCTCCTGTTCTCTAGTGAGTCTACCCCTGCACCGCTATGAG  
 2880 ATGTAGGTTATACTCGGGTGGAGGACAAGAGATCACTCAGATGGACGTGGCGATACTC

a Y I Q Y E P H L L F S S E S T L H R Y E -  
 2881 GTTCACCCATATGGGACCCCTCCAGTGGTCTGGCCAGAATTCAAACCACTCTCAGG  
 2940 CAAGTGGGTATAACCTGGGAGGGTCACCCAGGACCGGGCTTAAGTTTGGTGAGAGTCC

a V H P Y G T L P V G P G P E F K T T L R -  
 2941 ACTAACAAATGCAAGCTGCATAGTGCAGAACCTGACTGAACCCCCAGGCCACCTGTGCAT  
 3000 TGATTGTTACGTTGACGTATCACGTCTGGACTGACTTGGGGTCCGGGTGGACACGTA

a T N N A S C I V Q N L T E P P G P P V H -  
 3001 CCAGAGGAGCTTCAACACACAAACAGACTGAATGGGAGCAATACTCAGTGTCAAGTGGTG  
 3060 GGTCTCCCTCGAAGTTGTGTTGTCTGACTTACCCCTCGTTATGAGTCACAGTCCACCAC

a P E E L Q H T N R L N G S N T Q C Q V V -  
 3061 AGGTGCCACCTTGGGCAGCTGGCAAAGGGACTGAGGTCTCTGGACTATTGAGGCTG  
 3120 TCCACGGTGGAACCCGTCGACCGTTCCCTGACTCCAGAGACAACCTGATAACTCCGAC

48

a R C H L G Q L A K G T E V S V G L L R L -  
 GTTCACAATGAATTTCGAGAGCCAAGTTCAAGTCCCTGACGGTGGTCAGCACCTT  
 3121 -----+-----+-----+-----+-----+-----+-----+ 3180  
 CAAGTGTACTTAAAAGCTCTCGTTCAAGTCAGGGACTGCCACCAGTCGTGGAAA

a V H N E F F R R A K F K S L T V V S T F -  
 GAGCTGGGAACCGAAGAGGGCAGTGTCTACAGCTGACTGAAGCCTCCGGAGTGAG  
 3181 -----+-----+-----+-----+-----+-----+-----+ 3240  
 CTCGACCCCTGGCTCTCCGTCACAGGATGTCGACTGACTTCGGAGGGAACCTCACTC

a E L G T E E G S V L Q L T E A S R W S E -  
 AGCCTCTGGAGGTGGTCAGACCCGGCTATCCTCATCTCCCTGTGGATCCTCATAGGC  
 3241 -----+-----+-----+-----+-----+-----+-----+ 3300  
 TCGGAGAACCTCCACCAAGTCTGGCCGGATAGGAGTAGAGGGACACCTAGGAGTATCCG

a S L L E V V Q T R P I L I S L W I L I G -  
 AGTGTCTGGGAGGGTTGCTCTGCTCTCCTGTCTCTGCCTGTGGAAGCTTGGC  
 3301 -----+-----+-----+-----+-----+-----+-----+ 3360  
 TCACAGGACCCCTCCAAACGAGGACGAACGAGAGAACAGAACGGACACCTCGAACCG

a S V L G G L L L A L L V F C L W K L G -  
 TTCTTGCCCCATAAGAAAATCCCTGAGGAAGAAAAAGAGAAGAGAAGTTGGAGCAATGA  
 3361 -----+-----+-----+-----+-----+-----+-----+ 3420  
 AAGAAACGGGTATTCTTTAGGGACTCCTCTTTCTCTCAACCTCGTTACT

a F F A H K K I P E E E K R E E K L E Q  
 ATGTAGAATAAGGGCTAGAAAGTCCCTGGCAGCTTCTCAAGAGACTTCGATAAAA  
 3421 -----+-----+-----+-----+-----+-----+-----+ 3480  
 TACATCTTATCCCAGATCTTCAGGAGGGACCGTCGAAAGAAGTCTCTGAACGTATT

AGCAGAGGTTGGGGCTCAGATGGACAAGAAGCCGCCTGGACTATCTCCCCAGACC  
 3481 -----+-----+-----+-----+-----+-----+-----+ 3540  
 TCGTCTCAAACCCCCGAGTCTACCTGTTCTCGCGGAGACCTGATAGAGGGTCTGG

AGCAGCCTGACTTGACTTTGAGTCCTAGGGATGCTGCTGGCTAGAGATGAGGCTTAC  
 3541 -----+-----+-----+-----+-----+-----+-----+ 3600  
 TCGTCGGACTGAACTCAGGATCCCTACGACGACCGATCTACTCCGAAATGG

TCAGACAAGAAGAGCTGGCACCAAAACTAGCCATGCTCCCACCCCTGCTTCCCTCC  
 3601 -----+-----+-----+-----+-----+-----+-----+ 3660  
 AGTCTGTTCTCTCGACCGTGGTTGATCGGTACGAGGGTGGAGACGAAGGGAGGAGG

TCGTGATCCTGGTCCATAGCCAACACTGGGCTTTGTTGGGTCTTTATCCCCAG  
 3661 -----+-----+-----+-----+-----+-----+-----+ 3720  
 AGCACTAGGACCAAGGTATCGGTGTGACCCGAAAACAAACCCAGGAAAATAGGGTC

GAATCAATAATTTTTGCCTAGGAAAAAAAAAGCGGCCGCAATTGATATCAAGCT  
 3721 -----+-----+-----+-----+-----+-----+-----+ 3779  
 CTTAGTTATTAACGGATCCTTTTTTCGCCGGCGTTAAGCTATAGTTCGA

49

## (2) INFORMATION FOR SEQ ID NO. 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143 base pairs
- (B) TYPE: nucleic acid and amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (iii) MOLECULAR TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (B) CELLTYPE: chondrocyte

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

NdeI

1 GGGGCATATGGTTCAGAACCTGGGTTGCTACGTTGTTCCGGTCTGATCATCTCCGCTCT  
1 CCCGTATAACCAAGTCTTGGACCCAACGATGCAACAAAGGCCAGACTAGTAGAGGCGAGA 60

b G H M V Q N L G C Y V V S G L I I S A L -

61 GCTGCCGGCTGGCTCACGGTGGTAACCTACCTCCTAACGCTTGTCAGGTTATCAGCGG  
61 CGACGGCCGACAACGAGTGCCACCATTGATGAAGGATTGAAACAGGGTCCAATAGTCGCC 120

b L P A V A H G G N Y F L S L S Q V I S G -

BamHI

121 CCTGGTGCCGGCGCGGATCCCCCCC  
121 GGACCACGGCGCCCTAGGGGGG 143

b L V P R G S P -

29 -05- 2000

Amended set

## CLAIMS

1. A recombinant or isolated collagen binding integrin subunit  $\alpha 10$  comprising essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having essentially the same biological activity.
2. A process of producing a recombinant integrin subunit  $\alpha 10$  comprising essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having essentially the same biological activity, which process comprises the steps of
- a) isolating a polynucleotide comprising a nucleotide sequence coding for an integrin subunit  $\alpha 10$ , or homologues or fragments thereof having essentially the same biological activity,
  - b) constructing an expression vector comprising the isolated polynucleotide,
  - c) transforming a host cell with said expression vector,
  - d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit  $\alpha 10$ , or homologues or fragments thereof having essentially the same biological activity, in said transformed host cell, and, optionally,
  - e) isolating the integrin subunit  $\alpha 10$ , or homologues or fragments thereof having essentially the same biological activity, from said transformed host cell or said culture medium.
3. A process of providing an integrin subunit  $\alpha 10$ , or homologues or fragments thereof having essentially the same biological activity, whereby said subunit is isolated from a cell in which it is naturally present.
4. An isolated polynucleotide comprising a nucleotide coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially the same

29 -05- 2000

biological activity, which polynucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or suitable parts thereof.

5. An isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially the same biological activity, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit 10  $\alpha 1$ .

6. A vector comprising a polynucleotide or oligonucleotide coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially the same biological acitivity, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof.

7. A vector comprising a polynucleotide or oligonucleotide which hybridises to a DNA or RNA coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

8. A cell containing the vector as defined in any 25 one of claims 6 and 7.

9. A cell generated by steps a) to d) of the process as defined in claim 2, in which a polynucleotide or oligonucleotide coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially 30 the same biological acitivity, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, has been stably integrated in the cell genome.

10. Binding entities having the capability of binding 35 specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence of SEQ ID No. 1 or SEQ ID No. 2, or to homologues or fragments thereof.

**AMENDED SHEET**

29 -05- 2000

52

11. Binding entities according to claim 10, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

5 12. Binding entities according to claim 10, which are polyclonal or monoclonal antibodies, or fragments thereof.

10 13. A recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and fragments thereof having essentially the same biological activity.

15 14. A recombinant or isolated integrin heterodimer according to claim 13, wherein the subunit  $\beta$  is  $\beta 1$ .

20 15. A process of producing a recombinant integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and fragments thereof having essentially the same biological activity, which process comprises the steps of

25 a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit  $\alpha 10$  of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit  $\beta$  of an integrin heterodimer, or polynucleotides or oligonucleotides coding for homologues or fragments thereof having essentially the same biological activity,

30 b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit  $\alpha 10$  optionally in combination with an expression vector comprising said isolated nucleotide coding for said subunit  $\beta$ ,

35 c) transforming a host cell with said expression vector or vectors,

29 -05- 2000

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-unit  $\beta$ , or homologues or fragments thereof having essentially the same biological activity, in said

5 transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having essentially the same biological activity, or the  $\alpha 10$  subunit thereof from said transformed host

10 cell or said culture medium.

16. A process of providing a integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having essentially the same

15 biological activity, whereby said integrin heterodimer is isolated from a cell in which it is naturally present.

17. A cell containing a first vector, said first vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\alpha 10$  of an integrin heterodimer, or

20 for homologues or parts thereof having essentially the same biological activity, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and a second vector, said second vector

25 comprising a polynucleotide or oligonucleotide coding for a subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof having essentially the same biological activity.

18. Binding entities having the capability of binding specifically to an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, or an subunit  $\alpha 10$  thereof, having essentially the same biological activity.

30 19. Binding entities according to claim 18, wherein the subunit  $\beta$  is  $\beta 1$ .

29 -05- 2000

54

20. Binding entities according to claim 18 or 19, which are chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

5 21. Binding entities according to claim 18 or 19, which are polyclonal or monoclonal antibodies

22. A fragment of the integrin subunit  $\alpha 10$ , which fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the  
10 spliced domain.

23. A fragment according to claim 22, which is a peptide comprising the amino acid sequence

KLGFFAHKKIPEEEKGREEKLEQ.

24. A fragment according to claim 22, which comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

25 25. A fragment according to claim 22, which is a peptide comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of  
20 SEQ ID No. 1.

26. A method of producing a fragment of the integrin subunit  $\alpha 10$  as defined in any one of claims 22-25, which method comprises a sequential addition of amino acids containing protective groups.

25 27. A polynucleotide or oligonucleotide coding for a fragment of the integrin subunit  $\alpha 10$  as defined in any one of claims 22-25.

28. Binding entities having the capability of binding specifically to a fragment of the human integrin sub-  
30 unit  $\alpha 10$  as defined in any one of claims 22-25.

29. Binding entities according to claim 28, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

35 30. Binding entities according to claim 28, which are polyclonal or monoclonal antibodies, or fragments thereof.

**AMENDED SHEET**

29 -05- 2000

31. An *in vitro* process of using an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or a

5 homologue or fragment of said integrin or subunit having essentially the same biologically activity, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

10 32. An *in vitro* process according to claim 31, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

15 33. An *in vitro* process according to claim 31, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

20 34. An *in vitro* process according to claim 31, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

35. An *in vitro* process according to claim 31, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

25 36. An *in vitro* process according to claim 31, whereby the subunit  $\beta$  is  $\beta 1$ .

30 37. An *in vitro* process according to claim 31, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

38. An *in vitro* process according to any one of claims 31-37, which process is used during pathological conditions involving said subunit  $\alpha 10$ .

35 39. An *in vitro* process according to claim 38, which pathological conditions comprise damage of cartilage.

29 -05- 2000

40. An *in vitro* process according to claim 38, which pathological conditions comprise trauma, rheumatoid arthritis and osteoarthritis.

5 41. An *in vitro* process according to any one of claims 31-37, which is a process for detecting the formation of cartilage during embryonal development.

42. An *in vitro* process according to any one of claims 31-37, which is a process for detecting physiological or therapeutic reparation of cartilage.

10 43. An *in vitro* process according to any one of claims 31-37, which is a process for selection and analysis, or for sorting, isolating or purification of chondrocytes.

15 44. An *in vitro* process according to any one of claims 31-37, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

20 45. A process according to any one of claims 31-37, which is a process for *in vitro* studies of differentiation of chondrocytes.

25 46. An *in vitro* process of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal 30 including human origin.

47. An *in vitro* process according to claim 46, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

35 48. An *in vitro* process according to claim 46, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

29 -05- 2000

49. An *in vitro* process according to claim 46, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

5 50. An *in vitro* process according to claim 46, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid No. 337 of SEQ ID No. 1.

10 51. An *in vitro* process according to claim 46, whereby the subunit  $\beta$  is  $\beta_1$ .

15 52. An *in vitro* process according to any one of claims 46-51, which is a process for detecting the presence of an integrin subunit  $\alpha_{10}$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin heterodimer comprising said subunit  $\alpha_{10}$  and a subunit  $\beta$ , or of homologues or fragments thereof having essentially the same biological activity.

20 53. An *in vitro* process according to any one of claims 46-51, which process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

25 54. An *in vitro* process for detecting the presence of a integrin subunit  $\alpha_{10}$ , or of a homologue or fragment of said integrin subunit having essentially the same biological activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha_1$ .

30 55. An *in vitro* process according to claim 54, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

35 56. An *in vitro* process according to claim 54, whereby said fragment is a peptide chosen from the group

comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

57. An *in vitro* process according to claim 54, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

58. An *in vitro* process according to claim 54, whereby said fragment comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

10 59. An *in vitro* process according to claim 54, whereby said fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1.

15 60. An *in vitro* process according to any one of claims 54-59, which is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage.

20 61. An *in vitro* process according to claim 60, wherein the pathological conditions are any pathological conditions involving the integrin subunit  $\alpha 10$ .

62. An *in vitro* process according to claim 61, whereby said pathological conditions are rheumatoid arthritis, osteoarthritis or cancer.

25 63. An *in vitro* process according to claim 60, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

30 64. An *in vitro* process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

29-05-2000

65. An *in vitro* process according to claim 64,  
whereby said polynucleotide or oligonucleotide is a  
polynucleotide or oligonucleotide coding for a peptide  
chosen from the group comprising peptides of the  
5 cytoplasmic domain, the I-domain and the spliced domain.

66. An *in vitro* process according to claim 65,  
whereby said polynucleotide or oligonucleotide is a  
polynucleotide or oligonucleotide coding for a peptide  
comprising the amino acid sequence

10 KLGFFAHKKIPEEEKREEKLEQ.

67. An *in vitro* process according to claim 65,  
whereby said peptide comprises the amino acid sequence  
from about amino acid no. 952 to about amino acid no. 986  
of SEQ ID No. 1.

15 68. An *in vitro* process according to claim 65,  
whereby said peptide comprises the amino acid sequence  
from about amino acid no. 140 to about amino acid no. 337  
of SEQ ID No. 1.

20 69. An *in vitro* process according to claim 65,  
whereby said pathological conditions are any pathological  
conditions involving the integrin subunit  $\alpha 10$ .

70. An *in vitro* process according to claim 69,  
whereby said pathological conditions are rheumatoid  
arthritis, osteoarthritis or cancer.

25 71. An *in vitro* process according to claim 69,  
whereby said pathological conditions are atherosclerosis  
or inflammation.

30 72. An *in vitro* process according to any one of  
claims 64-71, whereby said cells are chosen from the  
group comprising chondrocytes, smooth muscle cells,  
endothelial cells, osteoblasts and fibroblasts.

35 73. A pharmaceutical composition comprising as an  
active ingredient a pharmaceutical agent or an antibody  
which is capable of using an integrin heterodimer com-  
prising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$   
thereof, or a homologue or fragment of said integrin or

29 -05- 2000

60

subunit  $\alpha 10$  having essentially the same biological activity, as a target molecule.

74. A pharmaceutical composition according to claim 73, for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels.

75. A pharmaceutical composition according to claim 73, for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

76. A vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-unit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$ , or DNA or RNA coding for said integrin subunit  $\alpha 10$ .

77. *In vitro* use of the integrin subunit  $\alpha 10$  as a marker or target in transplantation of cartilage or chondrocytes.

78. An *in vitro* method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

79. A method of *in vitro* detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-unit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit having essentially the same biological activity, with a sample, thereby causing said integrin, subunit  $\alpha 10$ , or homologue or fragment thereof, to modulate the binding to its natural ligand or other integrin binding proteins present in said sample.

**AMENDED SHEET**

29 -05- 2000

61

80. A method of *in vitro* studying consequences of the interaction of a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or 5 subunit having essentially the same biological activity, with an integrin binding entity and thereby initiate a cellular reaction.

81. A method according to claim 80, whereby the consequences of said interactions are measured as alterations 10 in cellular functions.

82. An *in vitro* method of using DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof as a target molecule.

83. An *in vitro* method according to claim 82, 15 whereby a polynucleotide or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit  $\alpha 10$ , or homologues or fragments thereof having essentially the same biological activity, and whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA 20 encoding an integrin subunit  $\alpha 1$ .

84. An *in vitro* method of using a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin 25 subunit  $\alpha 10$  or homologues or fragments thereof, as a marker or target molecule during angiogenesis.

85. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of stimulating cell surface expression 30 of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity.

86. A process of using a collagen binding integrin 35 subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or a

**AMENDED SHEET**

29 -05- 2000

homologue or fragment of said integrin or subunit having essentially the same biologically activity, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

87. A process according to claim 86, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

10 88. A process according to claim 86, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

15 89. A process according to claim 86, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

20 90. A process according to claim 86, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

91. A process according to claim 86, whereby the subunit  $\beta$  is  $\beta 1$ .

25 92. A process according to claim 86, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

93. A process according to any one of claims 86-92, which process is used during pathological conditions involving said subunit  $\alpha 10$ .

30 94. A process according to claim 93, which pathological conditions comprise damage of cartilage.

95. A process according to claim 93, which pathological conditions comprise trauma, rheumatoid arthritis and osteoarthritis.

35 96. A process according to any one of claims 86-92, which is a process for detecting the formation of cartilage during embryonal development.

29 -05- 2000

63

97. A process according to any one of claims 86-92, which is a process for detecting physiological or therapeutic reparation of cartilage.

98. A process according to any one of claims 86-92,  
5 which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

99. A process of using binding entities having the capability of binding specifically to an integrin subunit  
10  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same activity, as markers or target molecules of cells or  
15 tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

100. A process according to claim 99, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the  
20 spliced domain.

101. A process according to claim 99, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

102. A process according to claim 99, whereby said  
25 fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

103. A process according to claim 99, whereby said fragment comprises the amino acid sequence from about  
30 amino acid no. 140 to about amino acid No. 337 of SEQ ID No. 1.

104. A process according to claim 99, whereby the subunit  $\beta$  is  $\beta 1$ .

105. A process according to any one of claims 99-  
35 104, which is a process for detecting the presence of an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin

**AMENDED SHEET**

29 -05- 2000

heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or of homologues or fragments thereof having essentially the same biologically activity.

106. A process according to any one of claims 99-  
5 104, which process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

107. A process for detecting the presence of an integrin subunit  $\alpha 10$ , or of a homologue or fragment of  
10 said integrin subunit having essentially the same activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide shown in SEQ ID No. 1 is used as a marker under hybridisation conditions  
15 wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

108. A process according to claim 107, whereby said cells are chosen from the group comprising chondrocytes,  
20 smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

109. A process according to claim 107, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the  
25 spliced domain.

110. A process according to claim 107, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

111. A process according to claim 107, whereby said fragment comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of  
30 SEQ ID No. 1.

112. A process according to claim 107, whereby said fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of  
35 SEQ ID No. 1.

29 -05- 2000

113. A process according to any one of claims 107-  
112, which is a process for determining the  
differentiation-state of cells during development, in  
pathological conditions, in tissue regeneration or in  
therapeutic and physiological reparation of cartilage.  
5

114. A process according to claim 113, wherein the  
pathological conditions are any pathological conditions  
involving the integrin subunit  $\alpha 10$ .

115. A process according to claim 113, whereby said  
10 pathological conditions are rheumatoid arthritis, osteo-  
arthrosis or cancer.

116. A process according to claim 113, whereby said  
cells are chosen from the group comprising chondrocytes,  
smooth muscle cells, endothelial cells, osteoblasts and  
15 fibroblasts.

117. A process for determining the differentiation-  
state of cells during development, in pathological con-  
ditions, in tissue regeneration and in therapeutic and  
physiological reparation of cartilage, whereby a poly-  
20 nucleotide or oligonucleotide chosen from the nucleotide  
sequence shown in SEQ ID No. 1 is used as a marker under  
hybridisation conditions wherein said polynucleotide or  
oligonucleotide fails to hybridise to a DNA or RNA encod-  
ing an integrin subunit  $\alpha 1$ .

118. A process according to claim 117, whereby said  
polynucleotide or oligonucleotide is a polynucleotide or  
oligonucleotide coding for a peptide chosen from the  
group comprising peptides of the cytoplasmic domain, the  
I-domain and the spliced domain.  
25

119. A process according to claim 117, whereby said  
polynucleotide or oligonucleotide is a polynucleotide or  
oligonucleotide coding for a peptide comprising the amino  
acid sequence KLGFFAHKKIPEEEKREEKLEQ.  
30

120. A process according to claim 117, whereby said  
35 polynucleotide or oligonucleotide is a polynucleotide or  
oligonucleotide coding for a peptide comprising the amino

29 -05- 2000

66

acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

121. A process according to claim 117, whereby said polynucleotide or oligonucleotide is a polynucleotide or 5 oligonucleotide coding for a peptide comprising the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

122. A process according to claim 117, whereby said pathological conditions are any pathological conditions 10 involving the integrin subunit  $\alpha 10$ .

123. A process according to claim 117, whereby said pathological conditions are rheumatoid arthritis, osteo-arthrosis or cancer.

124. A process according to claim 117, whereby said 15 pathological conditions are atherosclerosis or inflammation.

125. A process according to any one of claims 117-124, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial 20 cells, osteoblasts and fibroblasts.

126. A method of using an integrin subunit  $\alpha 10$  as defined in claim 1 as a marker or target in transplantation of cartilage or chondrocytes.

127. A method of using binding entities having the 25 capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same 30 biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

128. Use of an integrin heterodimer comprising an 35 integrin subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity, as a target for anti-adhesive drugs or

**AMENDED SHEET**

29-05-2000

molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

129. A method of stimulating, inhibiting or blocking  
5 the formation of cartilage or bone, comprising admini-  
stration to a subject a suitable amount of a pharmaceuti-  
cal agent or an antibody which is capable of using an  
integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-  
unit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or  
10 fragment of said integrin or subunit  $\alpha 10$  having  
essentially the same biological activity, as a target  
molecule.

130. A method of preventing adhesion between tendon/  
ligaments and the surrounding tissue after infection,  
15 inflammation and after surgical intervention where adhe-  
sion impairs the function of the tissue, comprising admini-  
stration to a subject a suitable amount of a pharmaceuti-  
cal agent or an antibody which is capable of using an  
integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-  
unit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or  
20 fragment of said integrin or subunit  $\alpha 10$  having  
essentially the same biological activity, as a target  
molecule.

131. A method of stimulating extracellular matrix  
25 synthesis and repair by activation or blockage of an  
integrin heterodimer comprising a subunit  $\alpha 10$  and a sub-  
unit  $\beta$ , or of the subunit  $\alpha 10$  thereof, or of a homologue  
or fragment of said integrin or subunit  $\alpha 10$  having  
essentially the same biological activity.

30 132. A method of using DNA or RNA encoding an inte-  
grin subunit  $\alpha 10$  or homologues or fragments thereof as  
a target molecule.

133. A method according to claim 132, whereby a  
polynucleotide or oligonucleotide hybridises to the DNA  
35 or RNA encoding an integrin subunit  $\alpha 10$  or homologues or  
fragments thereof and whereby said polynucleotide or oli-

**AMENDED SHEET**

29 -05- 2000

gonucleotide fails to hybridise to a DNA or RNA encoding  
an integrin subunit  $\alpha 1$ .

134. A method of using a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit having essentially the same biological activity, or a DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof, as a marker or target molecule during angiogenesis.

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1/22

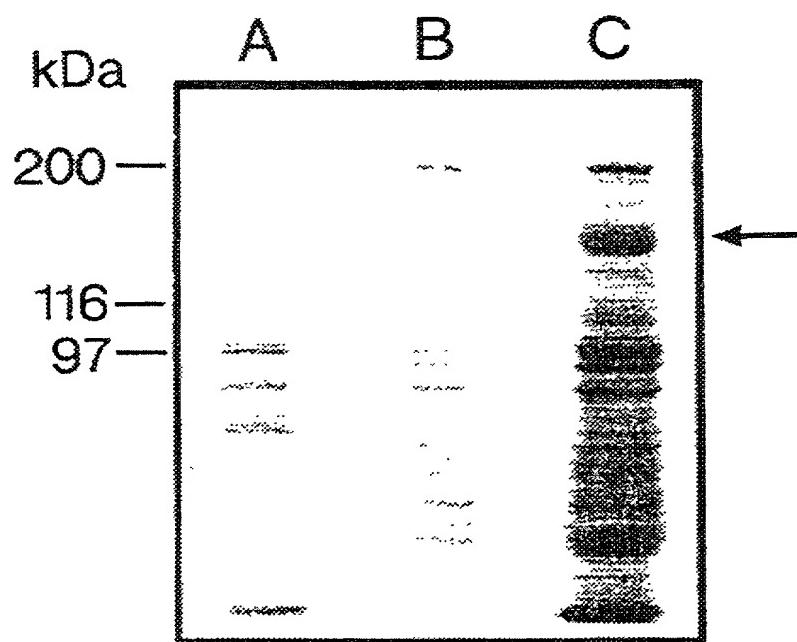


FIGURE 1

2/22

Peptide	Amino acid sequence
1	DNTAQTSAYIQYEPHHSI
2	GPGHWDR
3	AAFDGSGQR
4	FAMGALPD
5	FTASLDEWTTAAR
6	VDASFRPQGX LAP

FIGURE 2

3/22

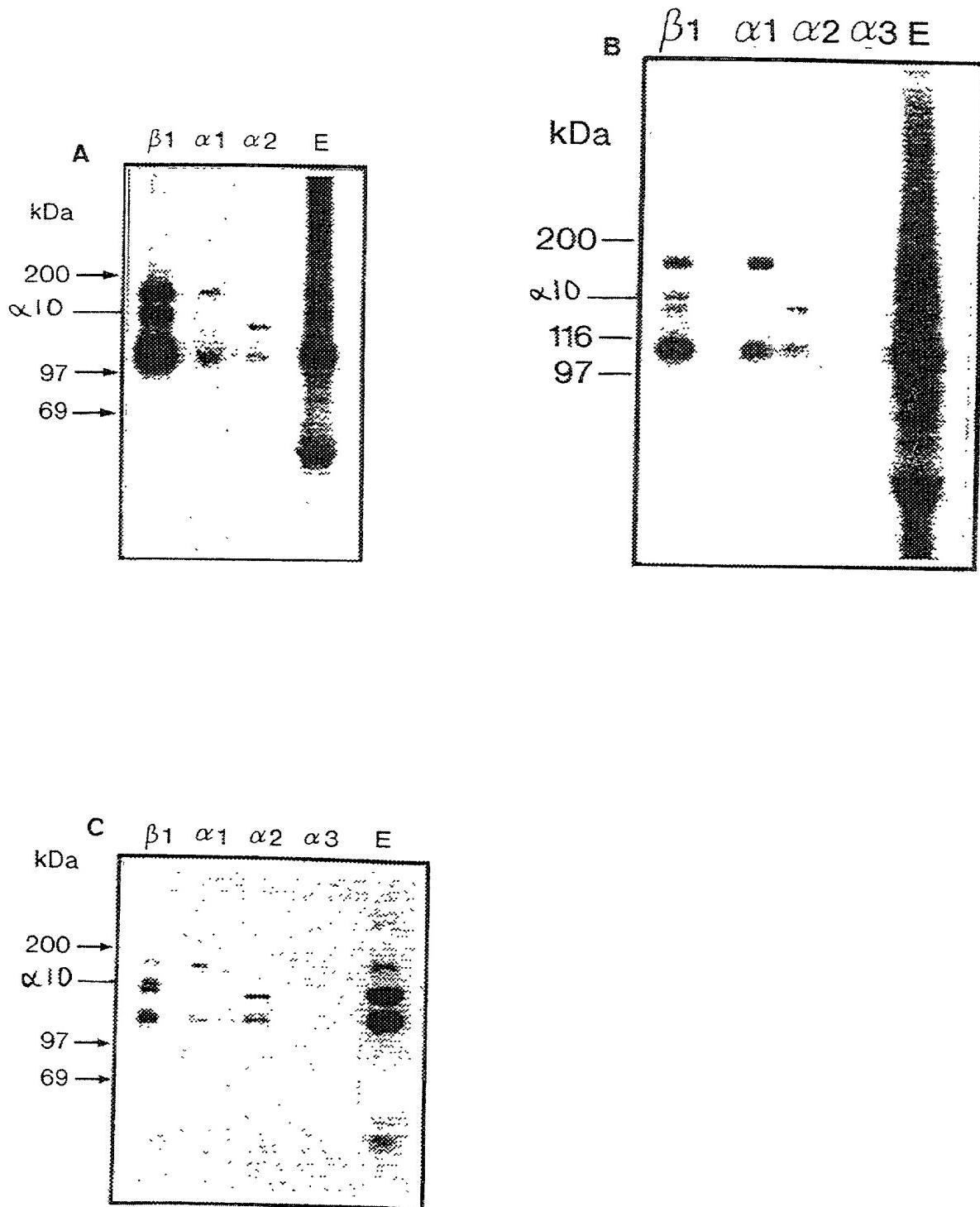


FIGURE 3

4/22

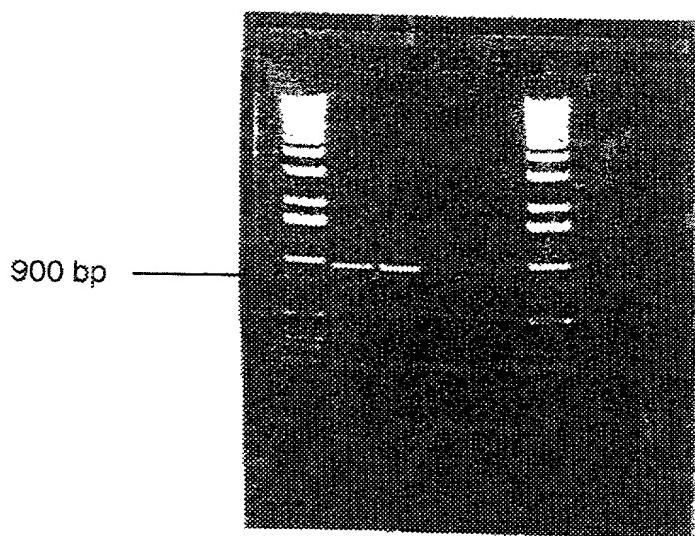


FIGURE 4

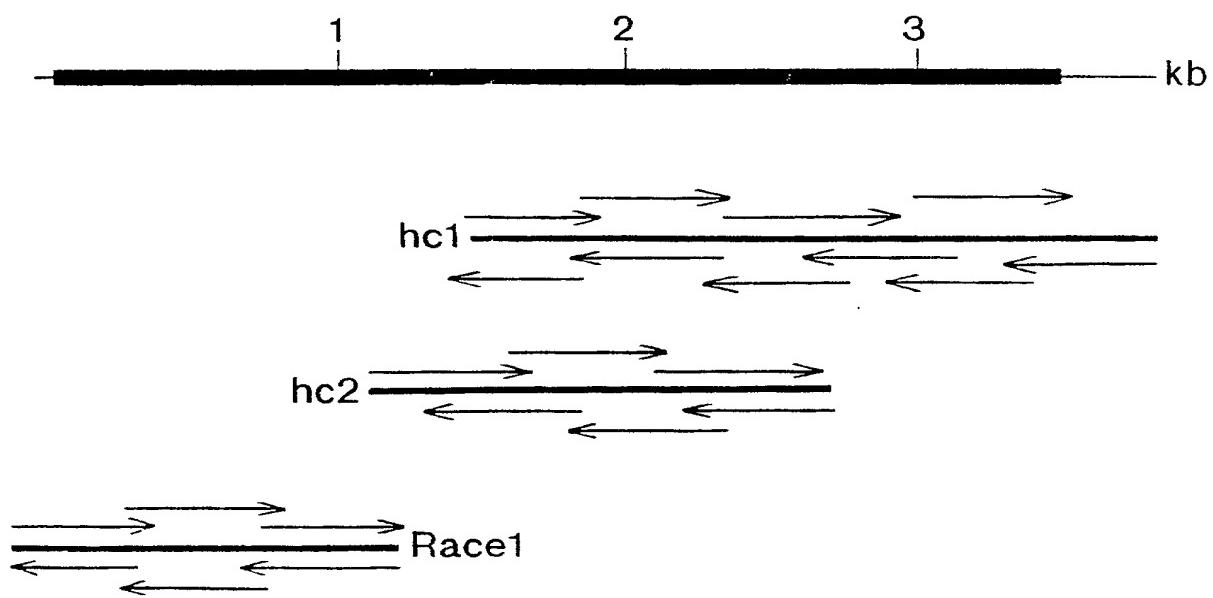


FIGURE 5

6/22

CAGGTCAAGAACCGATCAGGCATGGAACTCCCCCTCGTCACCTACCTGTTTGGCTTGTTTCCGAA	72		
M E L P P V T H L F L P L V F L T	-6		
GGCTCTGCTCCCCTTTAACCTGGATGAACTACCCACGCCATGCTCCAGGGCACCCAGAGGTAATT	144		
G L C S P F H L D E R H F R L F P P E A F	19		
GGATACAGTCTCTACAAACATGTTGGGGTGGACAGCGATGGATGCTGTTGGGCCCCCTGGATGGGCT	216		
G Y S V L O H V G G G Q R M H L V C A P W D G P	43		
TCAGGGCACCGGAGGGGGAGCTTACCTGCTGCTGGCCCTGAGGGGGGGCCACAAATGGCCAGGGC	288		
S G D R G D V Y T C P V G G A H A P C A K G	67		
CACTTACGACTACAACTGGGAAATCTCTACCTGCTGTTGGATGAACTGGGATCTCTCTGTTA	360		
H L G B Y O L G H S S H P A V R M H L G S L L *	91		
GAGACAGATGGTATGGGGGATTCATGGCTGTGCCCCCTCTGGCTGCTGCTGGCAGCTGCTCTC	432		
E T D G D G G H E A C A P L W S R A C G S S V F	115		
AGTTCGAGATACTGGCCGTTGGGATGCTGCTACAGGCTCGGGAGCTGGACCACCTGGCGG	504		
S S G H A D A S E P O Q G S D R P T A Q R	139		
TGCCCACATACATGGATGTTGTCATTGCTTGGATGCTGACAGCATCTACCCCTGGCTGAGTC	576		
C P T Y H D V V I V L D G S N S I Y P W S E V Q	163		
ACCTCTCTACGAAGACTGGTGGGAACTGTCTTATGACCCAGAACAGATACAGGTGGGACTGGTACAGTAT	648		
T E L R R L V G K L F I D P E Q I Q V G L V Q Y	187		
GGGGAGGCGCTTGACATGAGTGGTCCCTGGGAGATTCACGAGAACAGAGTGGAGAGCAGGAAAG	720		
G E S P V H E W S L G D E R T K E V V R A A K	211		
ACCTCTGGGGGGAGGGAGCAGAGAACAGACTGGCAACGAAATACTGGGGCTGCAAGAGANGGGTC	792		
H L S R R E G R E T K T A Q A I M V A C T E G E	235		
AGTCAGTCTGG	864		
S O S H G G R P E K A R L L V V V T D G E S H D	259		
GGAGAGGGCTTCTGAGCAACTAAAGGCTGAGGCTGAGCTGAGACCTGGATGGATGGCTGAGTC	936		
G E E P P A A L K A C E A G R V T R Y G I A V L	379		
GGTCACTACCTGG	1008		
G H Y L R R Q D P T S R E I R T A S D P	307		
GATGAGGGATCTCTCTACATGTCACAGATGGGGCTGCTGACTGACATGGGGGAGCTGGAGATGG	1080		
D E R F F F N V T D E A A L T D I V D A L G D R	331		
A T T T T G G C T G A A G G C C A T G C A G A A A A G G A A G C T C T T G G G A A T G T C T C A G A T T G G T T C	1152		
I F G L E G S H A H E S S E H E S S E G F	355		
TCCACTATGCCCTAAGGATGGCATCTTITGGGATGGGGGCTATGACTGGGGGCTCTGTC	1224		
S T H R L K D G I L F G H V G A Y D W G G S V D	379		
TOCTTGGAGGGGGCACCCCTTCCCCACGGAAAGGGACTGGAGAGGACTCTGGCTGAG	1296		
W L E G G H R L F P P R M A L E D E F F P P A L Q	403		
AACCATGGCTTACCTGGGTTATCTGTTCTGCAIGCTTGGGGGGGGGGGGGGGGGGGGGGGG	1368		
M H A A Y L G Y S V S S M L L R G G R L F S	427		
GGGGCTCTGGATTAGACATGGAGAACATCCCTTCACTGGGGGGGGGGGGGGGGGGGGGGGG	1440		
G A P R F R H R G K V I A T Q L K K D G A V R V	451		
GGCCAGGGCTCCAGGG	1512		
A Q S L O G E Q I G S Y F G S E L C P L D T D R	475		
GATGGGAACTGATGCTTACTGTGCTGGCTCCCCCATGTTCTGGGACCCAGAACAGGAAACAGGAGT	1584		
D G T T D V L L V A A P H F L G F Q N K E T G R	499		
GTTATATGTTATCTGGTAGGGCAGCAGCTCTCTGGCTGACGGGGGGGGGGGGGGGGGGGGGG	1656		
V V V Y L V G Q O S L L T L O G T L O P E P P Q	523		
GATGCTCGGTTGGCTGGCAAGGGAGCTGCTGATGACGGGGGGGGGGGGGGGGGGGGGGGGGG	1728		
D A R F G F A H G A L P D L N Q D G F A D V A V	547		
GGGGGGCTCTGGAAAGATGCCACCCAGGGAGACACTGTAACATGGACCCAGGGAGTGGAGCTGG	1800		
G A P F L E D G H O G A L Y L Y H G T Q S G V R P	571		
CATCTTGCCAGAGGATTTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG	1872		
H P A Q R I A A A S M P H A L S Y F G R S V D G	595		
CGCTCTAGATCTGGATGGGATGATCTGGCTGAGTGTGCTGCTGCTGCTGCTGCTGCTGCTG	1944		
R L D L D G D D L V D V A V G A Q G A X I L S	619		
-----			
TCCCTGGCCCATGTCATGACCCCATCTGGAGGTGACCCACAGGGCATAGTGTGGTCAAGGGAC	2016		
S R P I V H L T P S L E V T P Q A I S V V Q R D	611		
TGTAGGGGGGAGGGGAAAGGG	2088		
C R R R G O E A V C L T A L C F Q V T S R T P	657		
CGCTCTGGATGACCAACATCTACATGAGGTGACCCACCTGCTGATGAACTGCTGGGGGGGGGG	2106		
G R W D H O F Y H R F T A S L D E M T A G A R A	691		
-----			
CGATTTGATGGCTGGGGAGGG	2232		
A F D G S G Q R L S P P R R L R L S V G N V T C E	713		
*-----			
CGCTCTGGCTGAGTCATGCTGGGAGCTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	2304		
Q L H F G H V P R V A L T V T F A L	739		
-----			
QACAATACAAAGCCAGGGCTGCTGCTGATGAGGGCTACCCACCTCTATACAAAAGCTGGTCCCTTC	2376		
D N T T K P G P V L H E G S P T S I O K L V F F	763		
*-----			
TCAAGGATGTTGGCCCTGACATGATGATGATGCTGAGCTGCTGCTGCTGCTGCTGCTGCTG	2448		
S K D C G P D N E C V T D L V L Q V H M D I R G	787		
-----			
TCCAGGAGTGGCCCTGACATGATGATGCTGAGCTGCTGCTGCTGCTGCTGCTGCTGCTG	2520		
S R K A P F V V P R V K L V S T T L E N R	811		
-----			
MAGGMAATGTTACAAATAAGGCTGAGCTGAGTATCCTCTCTAGAAACCTGGCCAGCTCTACTCT	2592		
K E H A Y N T L S L S I F S R L H L A S L T P	835		
*-----			
CAGAGAGAGGGCCCATAAGGGGAAATGGGGGAGTGGCCGCCCTCTGGCTCATGGGGCTCTGGAGTGGGGCAT	2664		
Q R E S P I K V E C A P S A H A R L C S V G H	859		
-----			
CTTGTCTTCAAGACTGGGAGGCAAGGTCAGCTTCTGCTGAGTTAGCTTCTGCTGCTGCTGCTG	2736		
P V P F O T G A K V T E S P C S S L L S	883		
-----			
CAGGGCTTGGAGGACTGACTGGAGGCTGAGCTGAGGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	2808		
O V F G K L T A S S D S L E R N R G T L O E N T A	907		
*-----			
CAACAGCTCAGCTACATCAATATGAGCCCTCTCTGCTGCTGAGCTGAGCTGAGCTGAGCTGAG	2880		
Q T S A Y I O Y E P H L L F S S E S T L H R Y Z	931		
-----			
CTTCACCATATGGGACCTCCCTGGCTCTGGCTGAGGCTGAGCTTCTGCTGCTGCTGCTGCTG	2952		
V H Y P G T L S E R K F T T L R V Q N L	955		
-----			
GGTCTGCTGAGTGGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTGAGCTG	3024		
G C Y V V S G L I S A L L P A V A H G G N T F	979		
-----			
CTATCACTCTCAAGCTCATACATCAACATGCAACACTGCTGAGGAGCTGAGCTGAGCTGAGCTG	3096		
L S L S O V I T H N A S C I V Q N L T Z P P G P	1003		
*-----			
CTCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3168		
P V H H P E L Q H T M R L N G S N T Q C V V R	1027		
*-----			
TCCACCTTGGGAGCTGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3240		
C H L G Q L A K G T E V S V G L L R L V N H E F	1051		
-----			
CTCCACCTGGGAGCTGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3312		
F R R A K E F K S L T V V S T F E L G T E E G S V	1075		
-----			
CTACACTGAGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3384		
L O L T E A S R N S E S L L V E V V O T R P I L I	1099		
-----			
TCCCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3456		
S L M I L I G S V L G G L L L A L L V F C L W	1123		
-----			
AAAGCTGGCTCTGGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3528		
K L G F F A H R K H E P E E K L E Q	1145		
-----			
TAGATATGGGCTTAGAAAGCTCCCTGGAGCTCTTCTGAGGAGCTGCTGAGGAGGAGGAGGAGG	3600		
GCTGAGATGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3672		
AGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3744		
CACCCCTGCTCTCTCTCTCTGAGTGGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3816		
TATCCCCAGGAATGATTAATTTTTTGGCTAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3884		

FIGURE 6

7/22

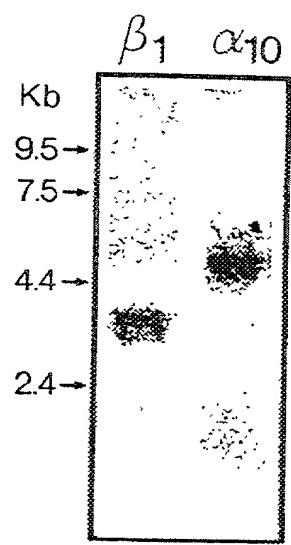
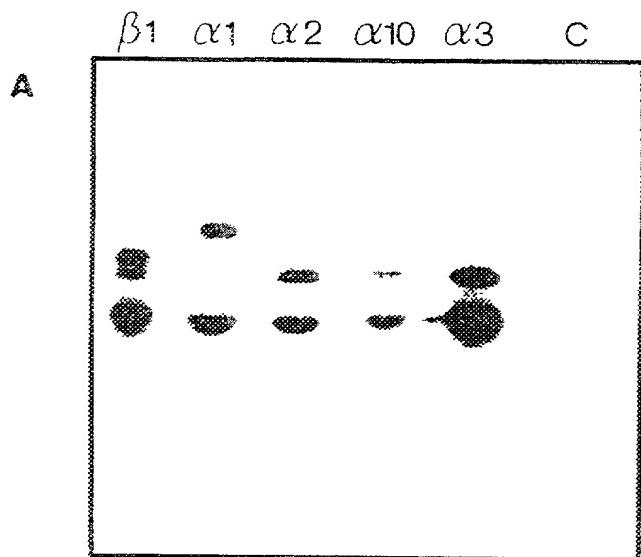


FIGURE 7

8/22



B IP:  $\alpha_{10}$   $\beta_1$   
Blot:  $\beta_1$   $\beta_1$

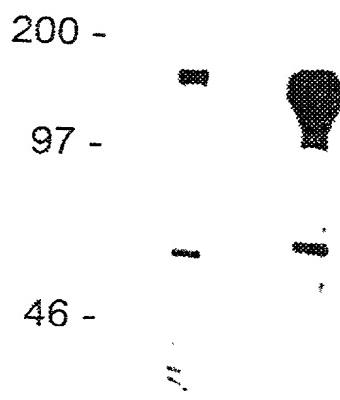


FIGURE 8

SUBSTITUTE SHEET (RULE 26)

9/22

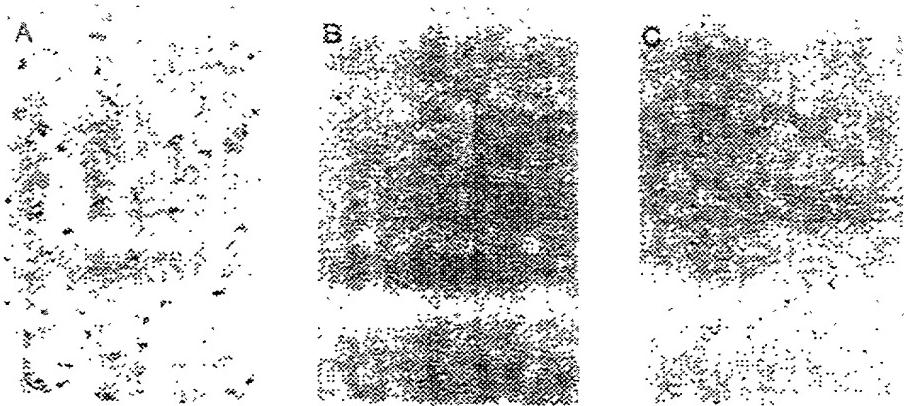


FIGURE 9

10/22

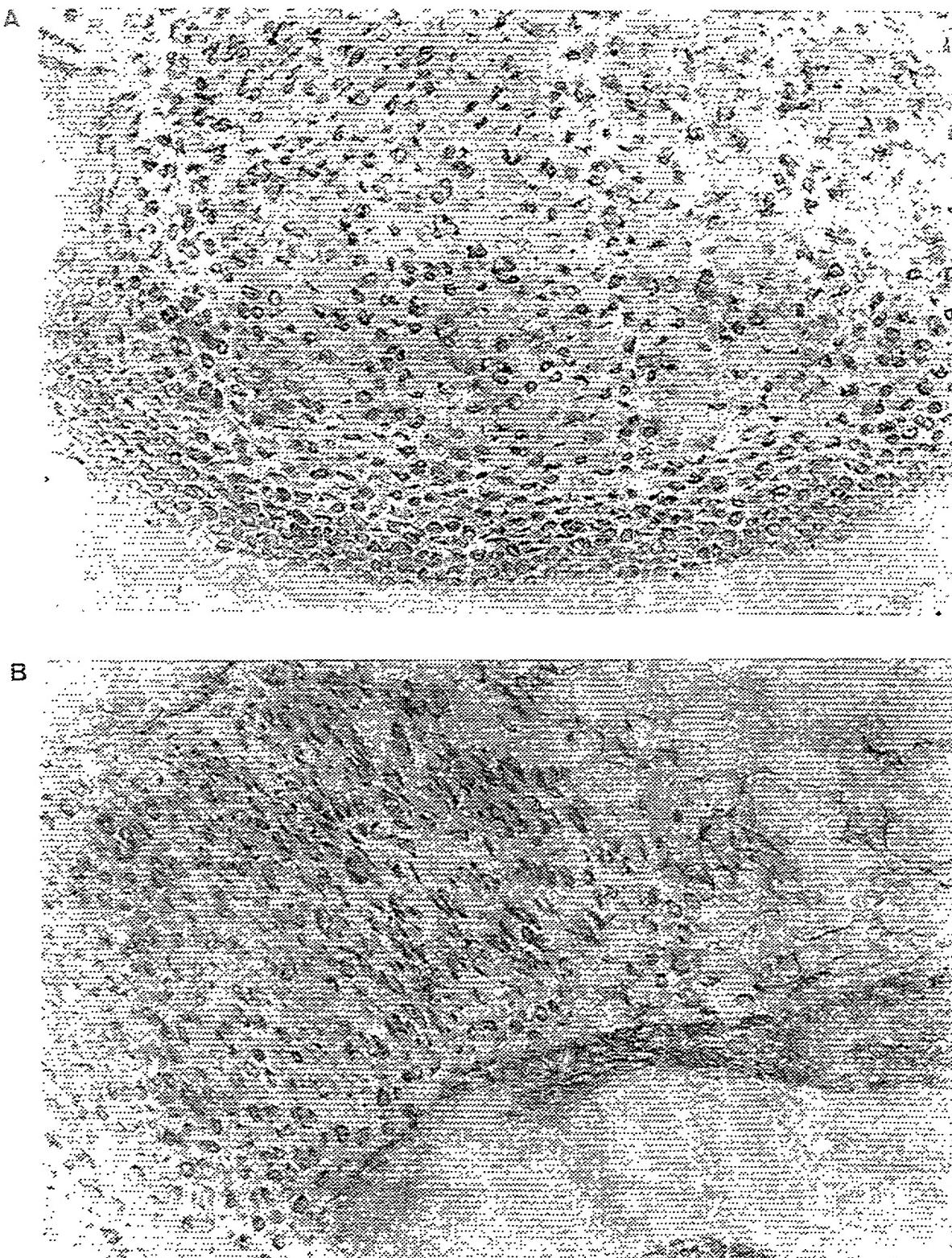
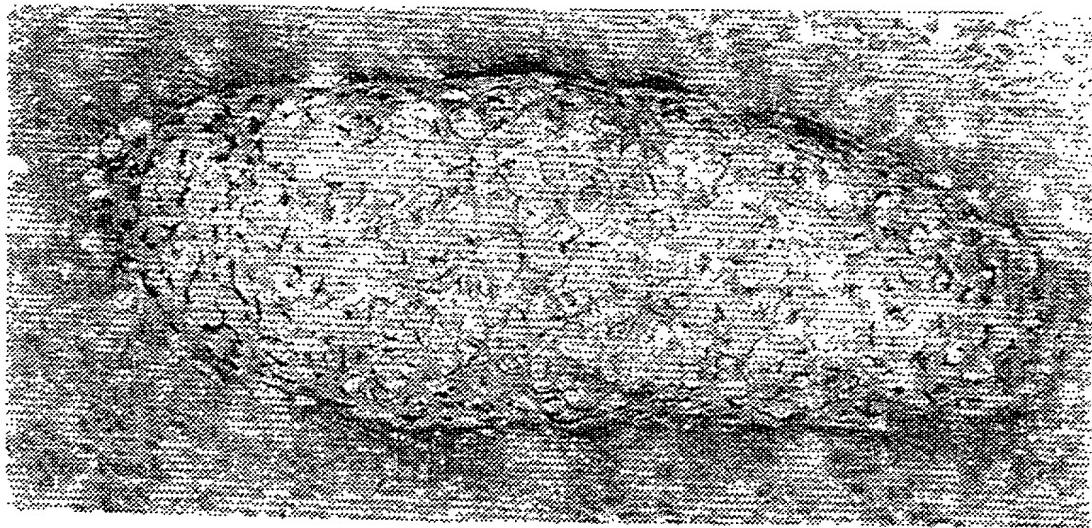


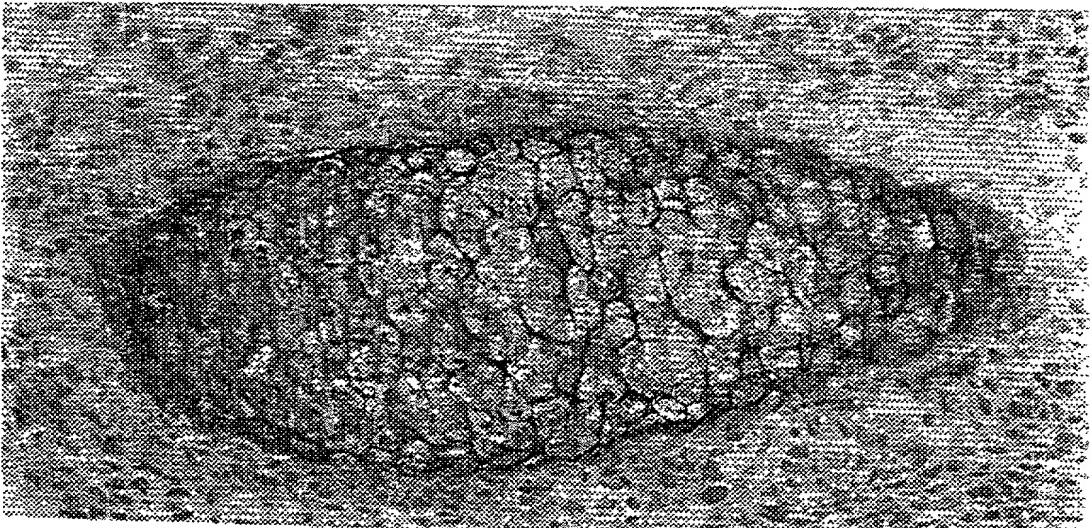
FIGURE 10  
**SUBSTITUTE SHEET (RULE 26)**

11/22

A



B



C

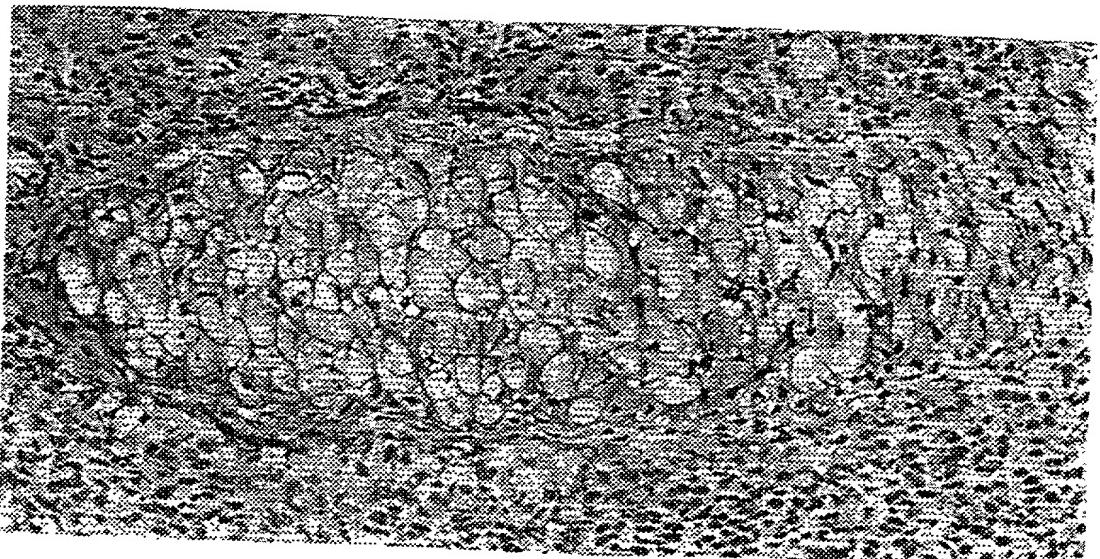


FIGURE 11

SUBSTITUTE SHEET (RULE 26)

12/22

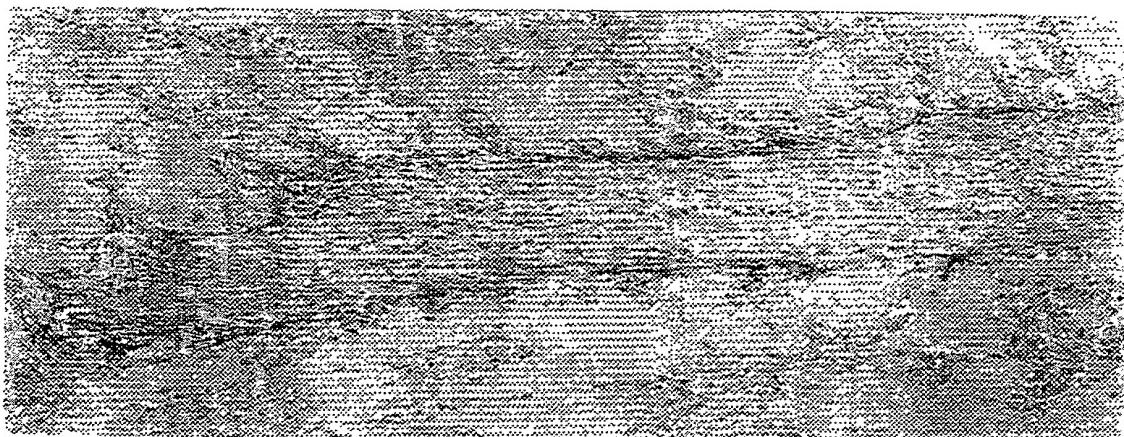
### Human RNA Master blot

Tissue	$\alpha 10$ expression	Tissue	$\alpha 10$ expression
Aorta	++++	Thyroid gland	-
Trachea	+	Salivary gland	-
Lung	++	Spleen	-
Fetal lung	++	Fetal spleen	-
Kidney	++	Thymus	-
Fetal kidney	(+)	Fetal thymus	-
Heart	(+)	Peripheral leucocyte	-
Fetal heart	++	Lymph node	-
Spinal cord	++	Appendix	-
Mammary gland	(+)	Placenta	-
Bone marrow	(+)	Whole brain	-
Small intestine	(+)	Fetal brain	-
Skeletal muscle	-	Amygdala	-
Liver	-	Caudate nucleus	-
Fetal liver	-	Cerebellum	-
Colon	-	Cerebral cortex	-
Bladder	-	Frontal lobe	-
Uterus	-	Hippocampus	-
Prostate	-	Medulla oblongata	-
Stomach	-	Occipital lobe	-
Testis	-	Putamen	-
Ovary	-	Substantia nigra	-
Pancreas	-	Temporal lobe	-
Pituitary gland	-	Thalamus	-
Adrenal gland	-	Subthalamic nucleus	-

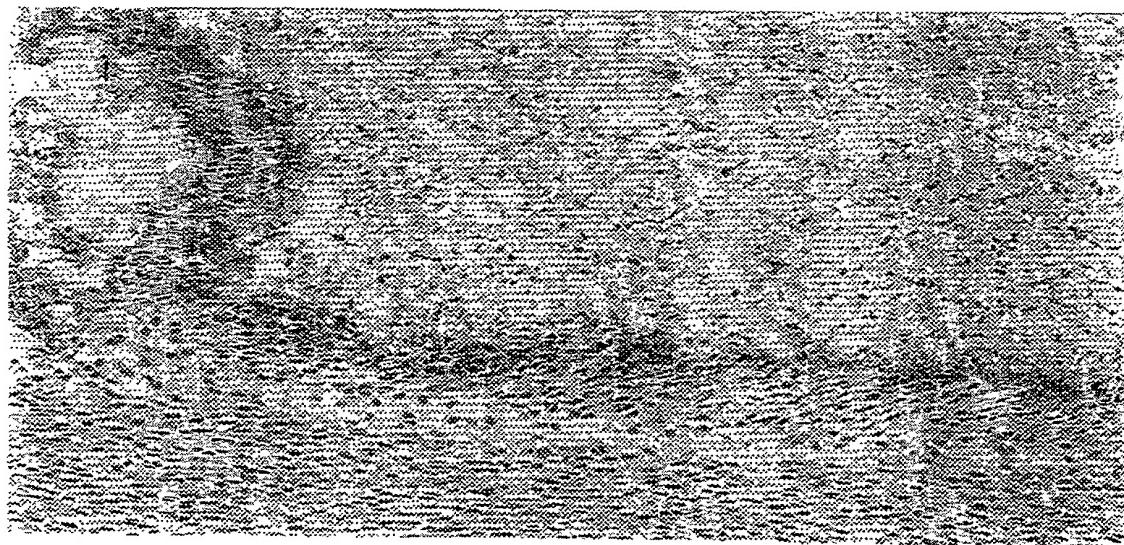
FIGURE 12

13/22

A



B



C

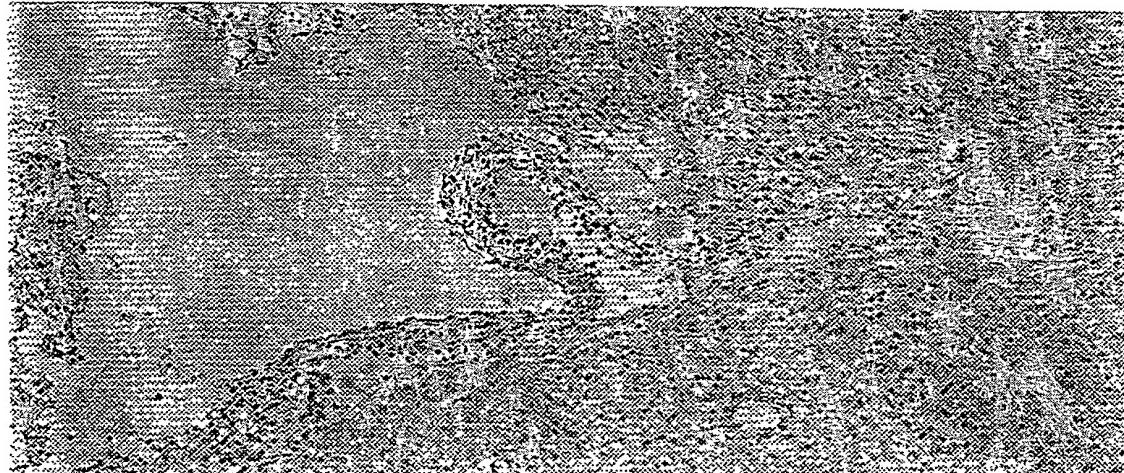


FIGURE 13

SUBSTITUTE SHEET (RULE 26)

14/22

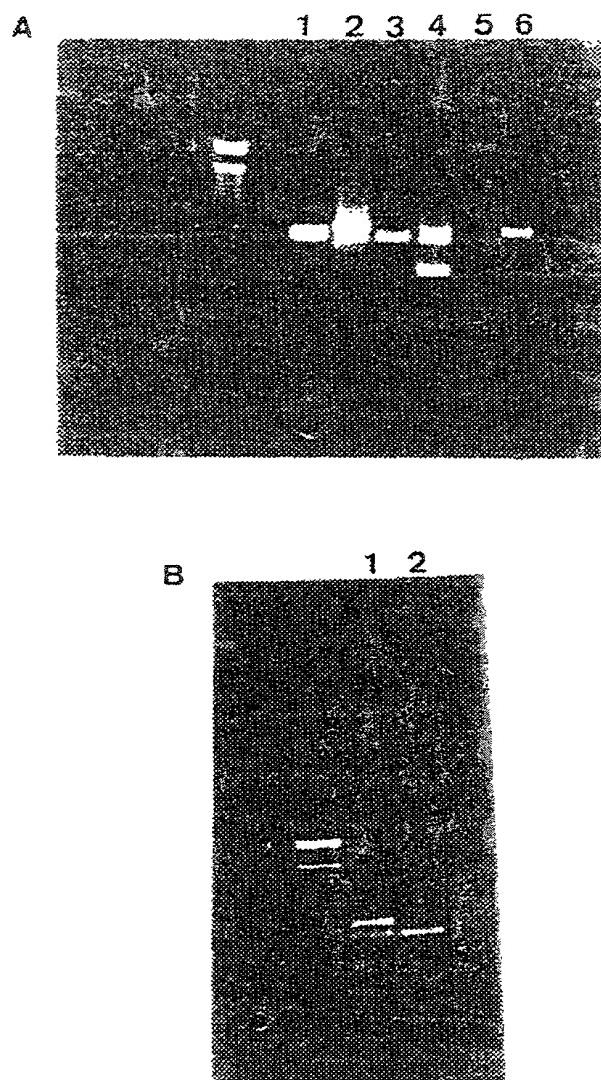


FIGURE 14

15/22

TGNTTMMKCMCACGAKMGWSAKGNCCGAKGGTKKGKVAAGTACARAGCTNGMIAAAARANGAAGTATGACCWGTGGC 80  
 ? ? ? ? R ? ? ? P ? V ? ? D ? A ? ? K ? X Y D ? W A  
 ? ? ? H ? ? ? R ? ? ? V T ? L ? K ? ? S M T ? G  
 V ? ? ? T ? ? ? G ? ? ? Q S ? ? K ? E V . P V G  
 CRAGATAGMKAMDAAGCNGMSAGKTRAMGGACGATGGNCMGCVAVGABWGNNAHTBCGGCNWDCARNGTCAAATK 160  
 ? ? I ? ? ? K ? ? ? G R W ? ? Q ? ? G ? ? G ? ? P N  
 P R . ? ? ? S ? ? ? D D G P A ? R ? ? ? A ? ? V O ?  
 ? D ? ? ? A ? ? . R T M ? ? P ? ? ? ? ? R ? ? ? S K ?  
 SANKTCSCAGGAACCHACCGAMTGGCTCGCARCCCDTAGGGATCAGGKACCATGRCCTS CCGRNSKACTCSGNKTGATWA 240  
 ? ? ? R N ? R ? G S Q P ? G I R ? D ? S P ? ? S ? . ?  
 ? ? ? G T ? G ? A R ? P - G S G T M ? ? R ? T ? ? D ?  
 ? S Q E P T ? W L A ? ? R D Q ? R ? L ? ? ? L ? ? I  
 ATCGMNWGTMCAGGCGHGGAAATTWAAAAGTANTGGTMGAMAKATGWGVMGGAATGATRRGTMGACTVTHVMGGVAK 320  
 I ? ? R ? A ? E L ? S ? G R ? M ? R ? ? V D ? ? G ?  
 S ? V G R R R N ? K V ? V ? ? ? G ? D ? ? T ? ?  
 N R ? ? ? G G G I ? K ? W ? ? ? ? M ? ? R L ? ? R ?  
 VTAKSGGTACAGGCGAACAKACRGAKGTGTCAGGAADTCAGNAGGACAAMMTTGCCGAAGTCMGGACTTAGKATRGAT 400  
 ? ? Y R R ? Q ? ? V - G ? Q ? D ? ? A E V R T . ? ?  
 ? , G T G E ? ? V S E E ? ? R T ? L P K S G L ? ? D  
 ? ? V Q A ? T ? ? C L R ? S ? G Q ? C R S ? D L ? ? I  
 ACGAANCKTRGATCTTAMADGGGGNKGCGAGTCGCGTAAACGVARATRGGSWGTCTACTTMAACNCCAAGNGDGGACA 480  
 Y E ? ? I L ? G G ? R V ? K R ? ? ? L L ? ? Q ? ? T  
 T ? ? S ? ? G ? ? S E C ? N ? ? ? V Y ? N ? K ? G H  
 R ? ? D L ? ? G ? A S A . T ? ? G ? S T ? T P ? ? D  
 TTTACTAGASGAGGAGAGTAGCCAGATCACDTGAGATGATCTAAGTGGGGTCCCGTTGCCAGTATATGAGAGGACTGGT 560  
 F T R ? G E - P D H ? R . S ? V G S R C Q Y M R G L V  
 L L ? E E S S Q I T - D D L ? W G P V A S I . E D W  
 I Y . ? R R V A R S ? E M I . ? G V P L P V Y E R T G  
 TCGGCAGACATWGATGCTCTTGCTGACTCACATATTGTCAGATGATCGATCTGWTGTCATCA 640  
 R Q T ? M L F A D S H I V A T ? M I R Y D L T S L I  
 F G R H ? C S L L T H I L L P . ? . S D T I ? C P S S  
 S A D I D A L C . L T Y C C ? E ? D Q I R S ? V P H H  
 TGAATSTGRGCGGTGATGCTAATGAGATTGGCTATGATGGAACAGAGACTTMTGCTACAGCAGGCCAATGAAGGTTTC 720  
 M N ? ? R D A N E I R L - W N K R L ? L Q Q A N E G F  
 - ? ? A V M L M R F A Y D G T R D ? C Y S R R M K V S  
 E ? ? P . C . . D S P M M E Q E T ? A T A G E . R F  
 TAGAGTAGGAGTCTCAGGAGGAGAGAACTGTGGACCTGGAGGACCCAGGGACTCCAGGAGGAAGTWGCCACAACIGGCTT 800  
 - S R S L R R R E T V D L E D Q G L Q E E V A T T G L  
 R V G V S G G E K L W T W R T R D D S L Q R R K ? P Q L A L  
 L E . E S Q E E R N C G P G P G T P G G G S ? H N W L  
 GMAGTTTCGGCTCCGATCTGATACTGGCTATGGCTCGTCCCTVGAGTTATCCCCTCTCTTGCTGGATGGCTCAGAAATGCCCTGG 880  
 ? F R L R S . Y ? L V L ? V I P L S C W M A Q K C L  
 ? S F G S D P D T G S S ? E L S P S L A G W L R N A W  
 ? V S A F I L I ? A R P ? S Y P P L L L D G S E M P G  
 ACCTTTCATCCCCACTGGACAAACTAGGGCTCTGGCTTGTGGCCCTGGGATTTGGGGCTGTGGCCCTCATATCCTC 960  
 D L F I P T G Q T R R L A L W P W D C G A V W P H I L  
 T F S S P L D K L G V W R C G P G I V G L C G L I S S  
 P F H P H W T N . A S G V V A L G L W G C V A S Y P  
 CATTCTGTCTATTCTCACCTTAATCTGCCCTGGNTACGACTCAAGCCYGAATGACAMTGTGGTACAAGATAAGGAGGG 1040  
 H S V Y S H P N L S L ? T T Q A ? T D ? V V Q D K E G  
 I L S I L T L I C P P W ? R L K P ? L T ? W Y K I R R  
 P F C L F S P . S V P G Y D S S P D . ? C G T R . G G  
 AGCCCAGGTGGGTGAGATGGAAGCTGAGATGGTCACTGTGTGCCMACCTCATGTAACTNCCTGACTGAAGTT 1120  
 A Q V G E M E A E M V H C V P T S L - F N ? L D . S  
 E P R W V R W K L R W ? T V C ? P H C N S T ? L T E V  
 S P G G . D G S . D G . L C A ? L I V I Q L P . L K L  
 AAAATCCAGATCCYTAGGGATGAGGGGAGAACCTGCCAAAGACGGGTCAGGAAGGCACTGCTAAGGAAGGCTCTGCA 1200  
 - N P D P - G . G E E F A K D G S G R Q C . G K A P A  
 K I Q I ? R D E K N L P T G Q E G S A K G R L L Q  
 K S R S L G M R G R T C Q R R V R K A V L R E G S C  
 GGCTCTGCAAGTGGACTTCATTCACTGGCTATGGCTCTGGGAGATATTAAAGGAGGGGGTGGGTACTT 1280  
 G L C S W T S F S P I A R I S . L F P L S L C L E S S  
 A S A V G L H S V P L P E S H S S S ? Y L S V L S L  
 R P L Q L D F I Q S H C Q N L I A L P ? I S L S . V.  
 TTAAGAATTGTTACCGGAGACAGAAATTCTCTTCTGACCTCTGGGAGATATTAAAGGAGGGGGTGGGTACTT 1360  
 - E F V T G D R I L F L S L L A R Y L K G G G W V T  
 V K N L L P E T E F S F L A S W P D I . K E G G G G L L  
 L R I C Y R R Q N S L S . P P G Q I F K R R G V G Y F

FIGURE 15a

16/22

FIGURE 15b

17/22

CAGGTGAGGGAAAGCAAACCTGGTTCTGGGAATGGAAGTATGTGATTGTTATAATTGGACCATTATGGCTAAA 2800  
 R . G K Q T W F L L G M E V M W I V Y N W D H Y G .  
 T G E G S K L G F C W E W K L C G L F I I G T I M A K  
 Q V R E A N L V S A G N G S Y V D C L . L G P L W L K  
 ATCTYCGGGGCGCTCAGGTCGGAGGTTAACCGATGCTATATTCCTGTCGACTCATGTTCAAGCACCCAAATGG 2880  
 N L A G A Q V G G . Y R C Y I S C V H S C S - T P K W  
 I ? R A L R S E V N T D A I F P V C T H V L R H P N G  
 S - T P K W Q R S G R R L I P M L Y F L C A L M F L D T Q M  
 CAGTGGCCAAAACCTCCTGGCTTGACCTCATTATCCTAAACCTTGTACCTAATTATCTAAACCTTGGTCTAAACT 2960  
 Q W P K L P L A C T S L S K P L Y L I T . N L G F K L  
 S G V Q N F L W L V P H Y L N L C T . L S K T V L W L N  
 A V A K T S S G L Y L I I . T F V P N Y L V P W S . T  
 CCACAGACATGAGGGCACAGAAAAGACGCTCTCATCTCCATTGGTTACACTGATTCTCACCTCCCTGCTTCT 3040  
 H R H E G T E K R R V S H L P F G Y T D S Y L F C F  
 S T D M R A Q K R D V V S L F H S V T L I R T F P A S  
 F Q T . G H R K E T C L S S S I R L H . F L P S L L  
 CCCTGCCATTGGTCTCTGGCTGAGGCATAATTGCTTACTATGGTCAGAACTCTGGTTCGCTAACGACCG 3120  
 S L P L V L L G A . G I I A L L C G Q N S G F A . R P  
 P C H W C S L V P E A . L P P Y Y V V T L G S P D R  
 P A I G A P C V L R H N C L T M W S E L W V R L T T  
 AGCTACAGTTCTGGCTCATAGCCCTGCAATTCTGGATTAAAAAAAAAAAAGGCTCACATATAAAAACCTTCTGA 3200  
 S Y S F W S H S P A N F L D . K K K A H I . N T F S E  
 S A T V S G L I A L P I S W I K K K R L T Y K I P F L  
 E L Q F L V S . P C Q F P G L K K V G S H I K Y L F .  
 AAATGAGCACAGTGTGAGTTAGATTGGGGATGGGGGTCTGGATGCAAGAGCAAGACAGTAGAGAGAAG 3280  
 N E H S V S - S - I L G D G G L L G C K E Q D S R E  
 K M S T V . V E V R F W G M E G C L D A K S K T V E K  
 K . A Q C E L K L D F G G W R V A W M Q R A R Q . R R  
 AGAATCATGGGAGGATAAGAGGCTGAATTTCCTGCTAGTGCCTATAATCTTCTTCTAAAATAACAGCTG 3360  
 E N H G R D K R L E F F P A S A L . S L E P K I T A L  
 R I M G G I R G W N F S L L V P Y N L C F L K . Q L .  
 E S W E G . E A G I F P C . C P I I F V S . N N S S  
 ATTTATGGGAATTGGGTCAAGGGAGAAAAGGAATCAGTAGGCACAGATGGACCCCAAGCGTGGACTAAAGTTGAGAAA 3440  
 I L W E L G S G E P N Q . A Q M G P Q A W T K V . G N  
 F Y G N W G Q E K G I S R R H W D P K R G L K F E E  
 D F M G I G V R R K E S V G T D G T P S V D . S L R K  
 CTATGGGAGTAGGCAAGGGGTGTTGTAAGGTGGATGAGATGACGGAGATTGTGGGGGGAGTCTGGGGGTGATAGG 3520  
 Y G S R Q G V F V R W M R . G D C G G G E S W G .  
 T M G V G K G C L . G G . D E E I V V G G S L G G D R  
 L W E . A R G V C K V D E M R R L W W G G V L G V I G  
 ACCCTTAACAGGGATAGATGCAAACCTGTGTGGGGCAGCCGGTGGTCCACCCACTTAATTAGCGTTGAGGTTGGCAG 3600  
 D P . Q G . M A N C V W A G R W F H P L N - R . G W Q  
 T L N R D R W Q T V C G Q A G G S T H L I S V E V G R  
 P L T G I D G K L C V G R P V V P F T . L A L R L A  
 GGCTGGAAGGAGGCCAGCACTCTAACCTGGAGAAAGTGCAGTGTGACAAGAAGAACAGAAAGAGGAGACACCCGGC 3680  
 G W K E P A L S T L E K V Q V . Q E E T E R G D T R A  
 A G R S Q H S Q P W R K C K C D K K K Q K E E T P P G  
 G L E G A S T L N L G E S A V T R R N R K R R H P G  
 AGGGAGCTCTTGCCATGTTCTCCATGGCCCTGGTTGGAGAATTAGGAAAGGGTGGTACTCTGCATCTCA 3760  
 G S S L P S F L P M A L A L G R I R K G W . L C I L  
 Q G A P C H R F F P W P W L W E E L G K G G G D S A S S  
 R E L L A I V S S H G P G F G K N . E R V V T L H P Q  
 GAAAAGCCCTCTCCCTCTTGACTCTCGAGGCTTAGAGAGGAGAAATGTGAGGAGAATGATGTGAAAGAGTAAC 3840  
 P K A L S P S L D S R G L E R R M C R R N D V E R V T  
 E K F P S L P L W T L E A . R G E C V G G M M W K E . L  
 K S P L S L F G L S R L R E E N V . E E . C G K S N  
 TGACCTATCCAGATGTGTCGTGAATGAGATTCAAGGAATGAGAAATACAGCTGTGCTTCAGCATGGCCGAGGGC 3920  
 . P I O M C L . M R F Q E . E W K Y S C A S A W P R A  
 D L S R C V C E . D F R N E E N G N T A V L Q H G R G  
 L T Y P D V S V N E I S G M R M E I Q L C F S M A E G  
 CCTTAGGATCCCTCACCCCCACGGAGAGAAATCATCCAACTCATCCCACCTGGGGTCTGAGGACATGACATTGAC 4000  
 L G S L T P T P Q E E N H P I I P F G V L R T . H .  
 P - D P S P P P P H R K R I I Q S H S L G F . G H D I D  
 L R I P H P H P T G R E S S N H F T W G S E D M T L T  
 ACAGAGCAGGAGAGCTGAGAGATAAACACTCCCTCTGCTTGTCTCCACTAAGCCTCACAGCTCTCATTAACGTGAT 4080  
 H R A G E L R . K H S L L S C L P L S L T S P S L T D  
 T E Q E S . D R N T P S C L V S H . A S P V U L H . L I  
 Q S P R A E I E T L P P V L S P T K P H O S F I N

FIGURE 15c

18/22

TGGTGGATGCTAATTATGATCCTCACCCCTCAGGTCTCTGCCTCCCCCTTAATCTGGATGAACACCACCCACGACTCTTC 4160  
 W W M L I M I L T F Q V S A F P L I W M N T T H D S S  
 G G C . L . S S P L R S L L P L . S G . T F P P T T L  
 L V D A N Y D P H P S G L C S P E N L D E H H P R L F  
 ACAGGGGCCACCAGAGGCCAATTGGATACAGTGTCTTACAGCATGTTGGGGGGTGACAGCGATGGTGGAGAGGGAAAACA 4240  
 Q G H Q R P N L D T V S Y S M L G V D S D G E R E N  
 H R A T R G R I W I Q C L T A C W G W T A M V R G K T  
 T G P P E A E F G Y S V L Q H V G E G O R W . E G K Q  
 GAGGACCGTGGGATCGGGACTATGCACTCACTGATAAAGGGGAGGACGGGCTCCAAGCTGGCTTTGAAAGTGGCTGGGC 4320  
 R G P W D R D Y A L T D K G E D R S K L A F E S A W G  
 E D R G I G T M H S L I K G R T G P S W P L K V P G A  
 P T V G S G L C T H . . R G G F V Q A G L . K C L G  
 TCCATGACGCTCTCATGCACTCTCCCTCTCACTATACTAAGGACCATGCTCACCGGATCTTATATCCATATTCTCCCTCC 4400  
 S M T S H A L S L S L Y . G P C S P D L Y I H I L L P  
 P . R L M H S P S H Y T K D H A H R I F I S I F S F  
 L H D V S C T L P L T I L R T M L T G S L Y P Y S F S  
 AGGATGCTGGTGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGGATGGG 4480  
 G C W W V P P G M G H Q V T G E G M F I V A L . G D  
 Q D A G G C P L G W A I R . P E R G C L S L L Y R G I  
 R M L V G A P W D G F S G D R R G D V Y R C S I J G G F  
 CCACAGTGTCCATGTACCAAGGCCACCTGGTAAGAAGAACCTGACCTTCCCCTGCTAATTCTGATGTTGACATC 4560  
 S T V L H V P K A T W V R R S L T F P L L T P D V D I  
 P Q C S H M Y Q R P P G . E E A . P F F P C . F L M L T S  
 H S A P C T K G H I G K K K P D L S P A N S . C . H  
 TAGTAACCTGTGACCCCTGGACCTTGCTTCAATGACCCCTGAACTAAAGAACCGAACATGACTTCATTCT 4640  
 . L . P L G P C L Q . P . T K E A E L . P H D F I L  
 S N S D P L D L V F N D P E L K K P N Y D P M T S F  
 L V T L T F W T L S S M T L N . R S R T M T P . L H S  
 CCTCTACCCCTCCCTCAACCGAGTGAECTATCACTGGAAATTCTCTCAGGCTGCTGTGAATATGCACCTAGGGATGTC 4720  
 F Y P S S N Q V T I N L E I P L S L L . I C T . G C  
 S S T L P P T R . L S T W K F L S A C C E Y A P R D V  
 L L P F L Q F G D Y Q L G N S S Q P A V N M H L G M S  
 TCTACTAGAGACAGATGCTGATGGGGATTATGGTGAGCTGAAAGAACGGGCTCAGAAGGTTCACAGCAGGGAAAGAG 4800  
 L Y . R Q M L M G D S W . A E R R A S E G S Q Q G R E  
 S T R D R C . W G I H G E L K E G P Q K V H S R E E S  
 L L E T D A D G G F M V S . K K G L R R F T A G K R  
 CATTATGGTATCTGGCAGTGGGGCTTGGGCTTTCATCCAGTGTCTGGAGGGCAGAGTCAGGGCTGATCTACAGAGT 4880  
 H Y G I W A V V A W A F H P S V L E A E S G L I Y R V  
 I M V S G Q W L L G P F I P V F W R Q S Q A . S T E  
 A L W Y L G S G G L G L S S Q C S G G R V R P D L Q S  
 GAGCTCCAGGACAGCCAAGGCTATGCAAGAGAAACCCCTGTTGAAAAACCCAAAACCAAACAAACAAACAAAC 4960  
 S S R T A K A M Q R N P V L K N P K P K L T K Q Q Q  
 - A P G Q P R L C R E T L F - K T Q N Q N . P N N N N  
 E L Q D S Q G Y A E K P C F E K P K T K T N Q T T T T  
 AGAAAAGCACCGTGGTAAGGGAAATTAGCTGCTAGAAGAGACAGGAATTCAAAACCCCTAGAGAGCAAGGCAGGGTT 5040  
 Q K K H R G K G N . S V . K R Q G I Q N P R E Q G R V  
 R K S T V V R E S L Y R R D K E F K T L E S K A G F  
 E K A P W . G K L V C I E E T R N S K F . R A R Q G  
 CCCCATGGAGTGTCTCCATCTCTTTAACTAGGTGTGTCGGAGAGGCCCTCTCACAGCCTGGGGATACTATTC 5120  
 P H G V V S I S L L T R C V F R E A L S S L G I T I S  
 P M E W S P F S L F . L G V C S E R P S Q A W G . L F S  
 S P W S G L H L S F N . V C V P R G P L K P G D N Y F  
 TTCTATCCCACCCAGGCCGTGCCCCCTTTGGTCTGCGCAGCTGCTCTCAGTCTGGAATATGIGCCCCGT 5200  
 P I H P G L C F S L V S C L R Q L C L Q F W N M C P  
 L L S T Q A C A P L W S R A C G S S U F S S G I C A R  
 S Y P P R P V F I F G L L V P A A A L S S V L E Y V P V  
 GTGGATGCTTCACTGGGCCCCAGGGAAAGCCGGCACCCACCGCCCACTGAGGCCAGTGGAGGGCCCTGGAGCTAG 5280  
 C G C F I P A P G K P G T H R P T . A S G R A L E A Q  
 V D A S F R P Q G S L A P T A Q R E P V E G P W K L S  
 W M L H S G P R E A W H P P P N V S Q W K G P G S S  
 TTCCCAAGATAGGGATGCTGGGTGGGAAAAACTAGGACAAGAACACTGGTGGAGGGTCTGCATGGCTATCTCATCATTCC 5360  
 F P D R D A G W E K L G Q R L G G G S A W L S S S F P  
 S Q I G M L G G K N . D K D L V E G L H G Y P H H S P  
 V F P . G C W V G K T R T K T W W R V C M A I L I I P  
 AAGTGTGCTTGCAGAAGAGGCCCTGTGTTGCTACTGATTAGAATTGACTCTTAGGAGAGGCCCTCAAGACACCAAGGAT 5440  
 S V L A E E A P V C . L I R I Q T P . E S L K T P G  
 Q V C L Q K R L L F A N . L S F R L L R R A S R H Q D  
 K C A C R R G S C L L T D . N S D S L G E P Q D T R I

FIGURE 15d

19/22

CTGGTTTACCAACTTAAAAACAAAACAGCATATCTGTGACAGCCTATCCCTATCCATCACGTGTCCAT 5520  
 S G F T N L K T K Q H S I S C A Q P I F H P S R V L H  
 L V L P T . K Q N K T A Y P V H S L S L I H H V S S I  
 W P Y Q L K N K T K Q H I L C T A Y P S S I T C P F  
 ATCTTATTTTGCGGCTTATAGATGCCAAGTCAGCACTCAGTATTGGTTCTCCCTCATGCCCTTCATACTTC 5600  
 I L F L W V L - M P S Q H S V I G F S P H A F H I L S  
 S Y F C G S Y R C Q V S T Q L L G S P L M P F I Y F  
 Y L I F V G L I D A K S A L S Y W V L P S C L S Y T F  
 TTATCTACTGCCCTTGGGAGATAGTCTTATGTAGGCCAGGCTGCTTGATCTGGAAATTGCTTGCTCAGCTTC 5680  
 Y L L P F G R . S Y V A Q A V L D L G I C L P Q L L  
 L I Y C L L G D S L M . P R L S L I L E F A C L S F S  
 L S T A F W E I V L C S F G C P . S W N L L A S A S Q  
 GTCTCAAGTACTGGGATAATAGGCATGGCTGCTGCCCTTGATGCCCTGTGGCCATTGGTAGGGCA 5760  
 S L K Y W D N R H A L S A W P L L N M P S V A I G R A  
 V S S T G I T G M H C L P G L C - T C P L W P L V G H  
 S Q V L G . . A C I V C L A F A E H A L C G H W . G  
 TGAGTCAAAATACTGCCCTCCCCAACACACACACAAACGAAAGTGAGGCTCTCTAATGTTCCATAGCACAGGGTAGT 5840  
 V K Y C P P P Q H T H K R K . G S L S V P . H R V V  
 E S N T A L P H N T H T N E S E A L . V F H S T G .  
 M S Q I L P S P T T H T Q T K V R L S K C S I A Q G S  
 GGAGGGCTCTCGCTAGTCATATTCTTACTCTGCCATCTCTCTTCTTGATTTCCACACTGGGACCTG 5920  
 V G L S L V H I S F F Y S A H L F F L . F P H W G F  
 W . A S R . C I F H S F T L P I S S F F D F H T G D L  
 G R P L A S A Y F I L L L C P S L L S L I S T L G T W  
 GCATAGTACTTCTCGTAATTAGAGAGAATTCCCTTTAAGTGCTGCATTCAGGCTCTGGACATTCTCC 6000  
 G I V L S W . L R E N S L L S A C I A A S S W D I L P  
 A . Y F P G N . E R I P F . V P A L L Q R P P G T F S L  
 H S T F L V I K R E F P F K C L H C S V L L G H S P  
 TGCTGACTACACCCACATCTTCATGTTTTGTTCCATCACTATGCCCTCTAGGCTGCCCACATACATGG 6080  
 C . L H F T S F H V F C F P S L C P P S R L S H I H G  
 A D Y T P H P S M F F V S H H Y A P P L L G C P T Y M  
 L L T P H I L P C F L F P I T M P P F . A V P H T W  
 ATGTCGTCATTGGATGGCTCAACAGTATCTATGCCAGACTTCAGACTTCTCGAGGGCTGGTAGGA 6160  
 C R H C F G W L Q Q Y L S L V R S S D F P S E A G R  
 D V V I V L D G S N S I Y P W S E V O T F L R R L V G  
 M S S L F W M A P T V S T P G Q K F R L S F G G W . E  
 AGACTGTCATCGATCCGGAGCAGATACAGGTAAGAGAAAGATATGTCATAGGATGGAGGGAAAGAAGTAACACTCC 6240  
 K T V H R S G A D T G K R K I C G . D W R E R S K H S  
 R L F I D P E Q I O V R E R Y V D R I G G K E V N T P  
 D C S S I R S R Y R . E K D M W I G L E G K K . T L  
 TGGACCCCTGGATGTAAGCGCCATGCCAGCCCTTGATGACACCCCTGGACATTGTTCTACAGAACTCATGCTCAA 6320  
 W T L G C K Q P C P A S . . H P G T L S S T E L M L K  
 G P L D V S S H V Q P L D D T L G H C L L Q N S C S  
 L D P W M . A A M S S L L M T P W D I V F Y R T H A Q  
 GAACTGTCATTAACCTACCAAAAAGTCACAAAATTCATAATGTTGAAGTAAGTTATGATTGTGGGGGGCCAC 6400  
 N C A I N L P K S H K N F I M F E V S L . L C G G G F  
 R T V Q L T Y Q K V T K I S . C L K . V Y D G V G G H  
 E L C N . L T K K S Q K F H N V . S K F M I V W G A T  
 ACTCAGAGCTCCCTTGTCTGTAGTTGGCTGGGCAATGCATGCCATGAGCTGCAAGTAGACACACCTGTTCACTT 6480  
 H S E L P F A A C S C L G N A C H E L Q V R H T C S L  
 T Q S F P L L L V A W A M H A M S C K L D T T P V H F  
 L R A S L C C L . L L G Q C M P . A A S . T H L F T  
 CCCCTTCATCGTGTGCAAGGGACACACCTGTTAGGGGTCACTTCCCTTCATCCTTGTGCTCCATCTCTACAG 6560  
 P L H R A A G G W T H L L G V H F P F I L C A P S S L R  
 P F I V L Q V G H T C . G F T S P S S F V L H L L Y  
 S P S C C R L D T P V R G S L P L H F L C S I F S T  
 CTCTTCATACATCCCATGTGGGACATGGTCTATTGTTCTCAGGTAGGACTGGTACAGTACGGGGAGAACCTGTGCATG 6640  
 S S Y I P C G H M V Y C S Q V G L V Q Y G E N P V H  
 A L H T S H V G T W S I V L R . D W Y S T G R T L C M  
 L F I H F M W A H G L L F S G R T G T V R G E P C A .  
 AGTGGTCCCTGGGAGACTTCGGAACAAAGGAAGAAGTGTGAGAGCAGCAAGGAACCTAAGTGGAGGGAGAACCTGTGCATG 6720  
 E W S L G D F R T K E E V V R A A R N L S R R E G R E  
 S G P W E T S E Q R K K L . E Q Q G T . V G G K G E K  
 V V P G R L P N K G R S C E S S K E P K S E G R A R  
 ACGAGAAACGCCCAAGCGATCATGGTGTGAGACATTGTAAAGGGGCGTGTGAGGGAGGGAGGAAGGATCAGCAG 6800  
 T P T A Q A I M V A W . D I V K G S C E G G G R I S R  
 R E P P F K R S W W H G E T L . R G R V R E E E G S A  
 N E N R P S D H G G M V R H C K G V V . G R R K D Q Q

FIGURE 15e

20/22

GGAGAGGGAGAGGGCTGGAGTGTAGTGTATACATCACAAAGATGCTCTGGCGCTTATCTTATCTGCATGCCAGAAGTT 6880  
 E R E R V W S V V Y T S Q D A L G A Y L Y L H A R S  
 G R G R G S G V . C I H H K M L W A L I F I C M P E V  
 G E G E G L E C S V Y I T R C S G R L S L S A C Q K F  
 CGTGGAGGAAGGCTAGGTTGTCACCATACTCTCTTACTGTATTGCAATTATGGTGTCTGGGTGTATCTCTC 6960  
 S W R K A R L L S P Y S L L L Y L H F M V S V G V S L  
 R G G R L G C C H H T L S Y C I C I L W C L W V Y L S  
 V E E G . V A V T I L S L T V F A F Y G V C G C I S  
 CTTGTCGTTCTGTTCTGCACACAGAACCTCCATCTTCTCTTACTCTCGCGTCAACTCTGATACTTAGCTCTCAA 7040  
 L V C S V S A H R T P S F L F Y S C V N S D T . L L N  
 L S V L F L H T E L H L S S S T P A S I L I P S F S  
 P C L F C F C T Q N S I F P L L L R Q F . Y L A S Q  
 CCACTCACGCCCTAGTATCTTCAAACATGACTCTAAACCTCTGGGGAGGCTACATGACCTGACTGCTCTTATTCTCC 7120  
 H S R P S I L F K H D S K P L G R L H D L T V F I L  
 T T H A L V F S N M T L N L W G G Y M T . L S L F S  
 P L T P . Y S F Q T . L . T S G E A T . P D C L Y S P  
 AGTTCTTGATCTTGCAACCCAAGTGTGTTGCTGAATGATCTATAAAATAAAATGCTGTACATATTACACTGATGA 7200  
 Q F L D L V N P S V C . M N L . I N N A C T Y L H . .  
 S S L I L S T Q V F A E . I Y K . I M L V H I Y T D D  
 V P . S C Q F K C L L N E S I N K . C L Y I F T L M  
 CAGATTATTTATGTTCCGTGCCATCTAACAGTCAGTTGACTCTGCCCCATGCTAGATACTGTTGG 7280  
 Q I I L L Y V C H C L N S Q V V T L C Q F A C . I L L G  
 R L F Y M F R A I . T V K L . L C A S L H A R Y C W  
 T D Y F I C S V P S K Q S S C D S V P V C M L D T V G  
 GAATGGTGTAGAAGACATCTGACCTCAGTGAACCTGCTGACAGTGTAAATACACTATACGGGCATGCCATGCAAGCCT 7360  
 N G V E D I . P Q . T A D S V N T L Y G H A C M Q A  
 G M V . K T S D L S E L L T V L I H Y T G M P A C K P  
 E W C R R H L T S V N C . Q C . Y T I R A C L H A S L  
 GTGTGTATGTCATGCATATGCACACACATATGACCATATAGCATTCTTATCTCTCTTAGCACAGAAGGGT 7440  
 C V Y V H A Y A H T Y I . P Y S I L L S L F L A Q K G  
 V C M C M H M H T H T Y D H I A F F Y L S S . H R R V  
 C V C A C I C T H I H M T I . H S F I S L L S T E G  
 TCAGTCAGTCCCAGGGGGGGACGACCAGAGGCCGCTAGGCTGCTGGTAGTTGTCACTGATGGAGAGTCCCAGATGGAGAG 7520  
 S V S P G G D D Q R P L G C W . L S L M E S P M M E R  
 Q S V P G G T T R G R . A A G S C H . W R V P . W R  
 E S Q S R G G R P E A A R L L V V V T D G E S H D G E  
 GAACCTCCAGCGCTAAAGGCTGTGAGGCTGGCAGAGTGACACGTTGGGATTGCGGTGAGACTTGATCAAGTCCA 7600  
 N F Q Q R . R P V R L A E . H V M G L R . D L I K S  
 G T S S A K G L . G W Q S D T L W D C G E T . S S P  
 E L P A A L K A C E A G R V T R Y G I A V R L D Q V Q  
 GTGTGTGTGTGTGTGTATCGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTGTGTAT 7680  
 S C F V L C C I V C V C V C V C V C V C V C V Y V . Y  
 V V L F C V V S C V C V C V C V C V C V C V C M C D M  
 L F C F V L Y R V C V C V C V C V C V C V C V I  
 GTGTGCATGCATCAGTCACATACCATAGTGTATATGGGGTCAGAGAACACCTCAGATGTTGGTCTCACCTTCCA 7760  
 V C M H Q C T Y H S V Y M R V R E Q P Q M L V L T F H  
 C A C I S A H T I V C I C G S E N N L R C W S S P S  
 C V H A S V H I F . C V Y A G Q R T T S D V G P H L P  
 TCTTGTCTCAAACCTGGATATCTTGTCACTCGGCATACAATAAGCCAGATTAGCTGACCCACAAGTCTGGGCAGGTCT 7840  
 L V P N W I S C S L R H T I S Q I S . P T S L G Q V  
 I L F Q T G Y L V H F G I Q . A R L A D P Q V L G R S  
 S C S K L D I L F T S A Y N K P D . L T H K S W A G L  
 TCTGTCGTTCCGCTCTGTCTTGGTTGAGGCATTCTGGAAATTACAGATAAGCTGATATCGAATTCTGCAGGCCGG 7920  
 F C L S L L S L G L R H S G I Y R . A . Y R I P A A R  
 S V S A S C L L V . G I L E F T D K L D I E F L Q P G  
 L S Q P P V S W F E A F W N L Q I S L I S N S C S P  
 GGGATCCACTAGTCTAGAGCGGGCCACCAAGGGAG 7958  
 G I H . F . S G R H Q G S  
 G S T S S R A A A T K G  
 G D P L V L E R P P P R E

FIGURE 15f

21/22

1      2      3      4

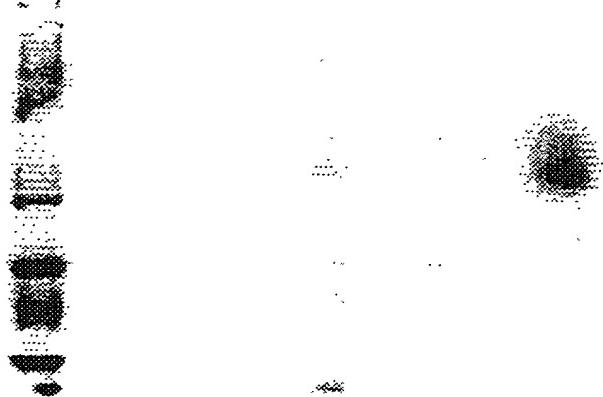


FIGURE 16

22/22

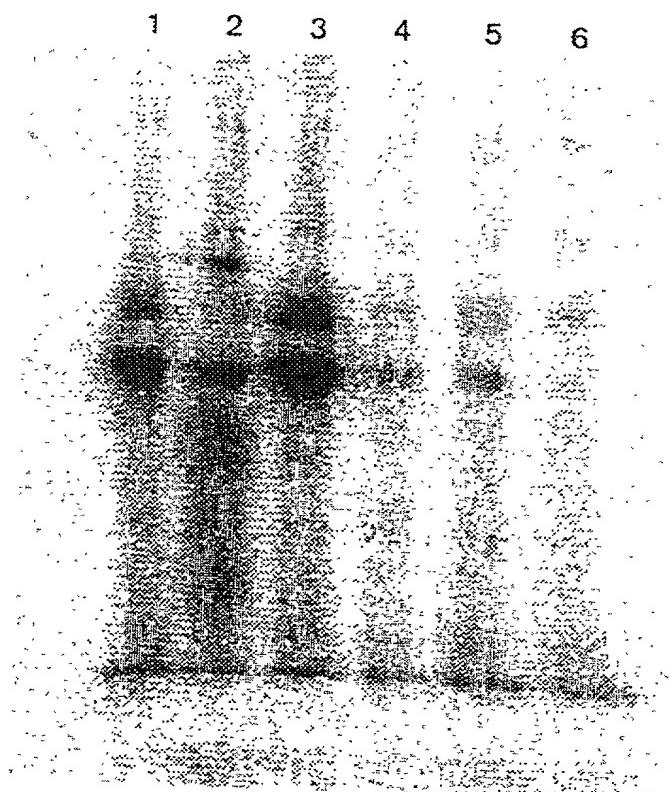


FIGURE 17

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY  
(Includes Reference to Provisional and PCT International Applications)**

Attorney's Docket No.  
003300-685

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;  
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**An integrin heterodimer and a subunit thereof**

the specification of which (check only one item below):

- is attached hereto.  
 was filed as United States application

Number \_\_\_\_\_  
on 2 October 2000  
and was amended  
on \_\_\_\_\_ (if applicable).

- was filed as PCT international application  
Number \_\_\_\_\_  
on \_\_\_\_\_  
and was amended  
on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:**

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9801164-6	2 April 1998	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Sweden	9900319-6	28 January 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED)**  
 (Includes Reference to Provisional and PCT International Applications)

Attorney's Docket No.  
 003300-685

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

**PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:**

U.S. APPLICATIONS		STATUS (check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.		
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)
PCT/SE99/00544	31 March 1999	

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	R. Danny Huntington	27,903	Gerald F. Swiss	30,113
Robert S. Swecker	19,885	Eric H. Weisblatt	30,505	Michael J. Ure	33,089
Platon N. Mandros	22,124	James W. Peterson	26,057	Charles F. Wieland III	33,096
Benton S. Duffett, Jr.	22,030	Teresa Stanek Rea	30,427	Bruce T. Wieder	33,815
Norman H. Stepno	22,716	Robert E. Krebs	25,885	Todd R. Walters	34,040
Ronald L. Grudziecki	24,970	William C. Rowland	30,888	Ronni S. Jillions	31,979
Frederick G. Michaud, Jr.	26,003	T. Gene Dillahunty	25,423	Harold R. Brown III	36,341
Alan E. Kopecki	25,813	Patrick C. Keane	32,858	Allen R. Baum	36,086
Regis E. Slutter	26,999	Bruce J. Boggs, Jr.	32,344	Steven M. du Bois	35,023
Samuel C. Miller, III	27,360	William H. Benz	25,952	Brian P. O'Shaughnessy	32,747
Robert G. Mukai	28,531	Peter K. Skiff	31,917		
George A. Hovanec, Jr.	28,223	Richard J. McGrath	29,195		
James A. LaBarre	28,632	Matthew L. Schneider	32,814		
E. Joseph Gess	28,510	Michael G. Savage	32,596		



**21839**

and: None

Address all correspondence to:



**21839**

Benton S. Duffett, Jr.  
 BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
 P.O. Box 1404  
 Alexandria, Virginia 22313-1404

Address all telephone calls to: Benton S. Duffett, Jr.

at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED)  
(Includes Reference to Provisional and PCT International Applications)ATTORNEY'S DOCKET NO.  
003300-685

FULL NAME OF SOLE OR FIRST INVENTOR <b>Evy Lundgren-Åkerlund</b>	SIGNATURE <i>Evy Lundgren-Åkerlund</i>	DATE 2000-10-09
RESIDENCE <b>Bjärred, Sweden</b>	CITIZENSHIP <b>Sweden</b>	
POST OFFICE ADDRESS <b>Trollsjövägen 165, 237 33 Bjärred, Sweden</b>		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		